

# **Cardiff School of Pharmacy & Pharmaceutical Sciences Research Abstracts**

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## FOREWORD

The School of Pharmacy and Pharmaceutical Sciences, Cardiff University, is the only school of pharmacy in Wales and is one of the top schools of pharmacy in the UK. Our students graduate well-prepared and satisfied, as seen by the consistently high pass rate in the pharmacist registration examination and high ranking in the National Student Survey respectively.

In addition to supporting individual pharmacists in their initial and ongoing education and development, the School is active in research that has been independently judged to be predominantly of international standing, more than half of which is recognised as world-leading or internationally excellent and with many interdisciplinary and external collaborators. Research at the School encompasses medicinal chemistry, drug delivery and microbiology, pharmacology and physiology, and pharmacy practice and clinical pharmacy, and it impacts on healthcare and pharmaceutical sciences throughout the UK and the world. Further information on the School's research activities and degree programmes, along with contact details for academic staff can be found at <http://www.cardiff.ac.uk/phrmy>.

At the Cardiff School of Pharmacy and Pharmaceutical Sciences, the combination of these strengths allows us to successfully deliver research-led learning and teaching. All of our MPharm students undertake a significant, independent Masters level research project in the final year of the four-year degree, and present and defend their research. The high numbers of well-qualified UK, EU and international students that we attract to our postgraduate diplomas and degrees also contribute significantly to our research output.

This is the 18<sup>th</sup> year in which we have published the abstracts of our students' research. Within this publication the student is the first named author, and collaborators and supervisors follow. An alphabetical list of authors appears in the index.

Many thanks to our colleagues for their assistance in collating this book.

***Dean Routledge, Rhys Thomas & Justine Jenkins***  
***July 2018***



## Technology in Community Pharmacies: The Perceptions of Pharmacists and the Community Pharmacy Team on current Patient Medication Record systems

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Community pharmacy services are continuously facing issues in the delivery of more efficient practice. It was suggested that smarter technology could be used to empower the pharmacist and allow them to provide better patient care.<sup>1</sup> There is a push for using swifter technology to bridge knowledge gaps in healthcare.<sup>2</sup> The major piece of technology used to support pharmacists in delivering pharmaceutical care is the Patient Medication Record (PMR) system, which is now in wider use.<sup>3</sup> Therefore, this study aims to explore the perceptions of pharmacists and the community pharmacy team on current PMR systems and identify key improvements that need to be implemented to support the community pharmacist in providing better quality care.

After attaining ethical approval, pharmacists were invited to take part in this study through purposive sampling. A topic guide was developed from the literature review and then trialled in the pilot study. Two focus groups were used to explore participant perceptions and were recorded via an audio recorder. Data was transcribed ad verbatim and analysed thematically.

Eleven participants were interviewed in the two focus groups. Two main themes were identified. The first theme being existing technology and encapsulated three sub-themes, namely: benefits of PMR systems, limitations of PMR systems and then other technologies. The second theme was challenges of community pharmacy and contained the following five sub-themes: funding cuts, how community pharmacists are perceived, lack of integration, unnecessary paperwork and embracing technology.

PMR systems were generally perceived as beneficial. However, their limitations have suggested that developments are needed to meet the needs of community pharmacists today. This study suggests that future PMR systems should be integrated with other healthcare professionals' systems, easy to use and ensure patient safety. In addition, system developers should make training and support more efficient to meet the needs of PMR system users.

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## The role of Prostaglandin E2 (PGE<sub>2</sub>) on keratinocyte growth rate

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Three-dimensional bio-printing of skin and organs using bio-printers has recently showed promising outcomes leading to a novel approach towards tissue based research.<sup>1</sup> A suitable candidate for printing is human skin, as current laboratory skin models have limited use especially in the study of skin cancer. PGE<sub>2</sub> has been implicated to enhance growth of skin tumours with observations showing overexpression of PGE<sub>2</sub> in those skin cells.<sup>2</sup> Thus, we conducted experiments aimed to test a range of PGE<sub>2</sub> concentrations and its effect on the increase of HaCaT cell (immortalised keratinocytes) proliferation rate. This will provide important observations of how PGE<sub>2</sub> effects the surrounding cells firstly in 2D before integration with future 3D models.

A two-dimensional monolayer culture of HaCaT cells in low calcium (basal-like) and high calcium (differentiated) concentration media were seeded into 12 well-plates at 2x10<sup>4</sup> per well in 6 wells and analysed over 6 days. PGE<sub>2</sub> was incorporated in the growth medium at concentrations of 1, 10, 50 and 100ng/ml, whilst a control group was analysed as a comparison.

From the results collected, the highest level of confluency and proliferation rate was evidently seen from concentration 10ng/ml of PGE<sub>2</sub> for both calcium levels (p=0.02). Results were highly significant for low calcium

but a non-significant trend was observed with the high calcium HaCaT cells. PGE<sub>2</sub> showed a fast onset with a long duration of action.

It can be confirmed PGE<sub>2</sub> is an important lipid mediator for keratinocyte proliferation and its production does affect neighbouring cells. The addition of concentrations higher than 10ng/ml of PGE<sub>2</sub> caused the death of cells via necrosis which could be due to desensitisation of prostaglandin receptors. In the future, the role of PGE<sub>2</sub> in cellular homeostasis would require analysis in a 3D-model for results representative of human skin.

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## Investigating the impact of the outer membrane and efflux pumps on the antimicrobial susceptibility profile of carbapenem-resistant Enterobacteria

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The aim of this project was to investigate the impact of the outer membrane and efflux pumps on the antimicrobial susceptibility profile of carbapenem-resistant Enterobacteria. *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella* spp, are Gram-negative bacteria normally found in the intestines.<sup>1</sup>

Fifty clinical isolates of *E. coli* & *K. pneumoniae* were prepared using R2A broth then tested against varying concentrations of chlorhexidine digluconate (CHX) alongside carbonyl cyanide 3-chlorophenylhydrazone (CCCP) or ethylenediaminetetraacetic acid (EDTA). Minimum Inhibitory Concentrations (MIC) were determined as the lowest concentration that inhibit visible growth. Minimum Bactericidal Concentrations (MBC) were determined by plating out the content of wells with no observable growth on TSA plates and incubation for 24h at 37C.

When comparing MICs of EDTA and CCCP alone, the average MIC using CCCP was higher (3.71 µg/ml) than EDTA (3.65 µg/ml), which shows at the concentrations used, EDTA was a more effective adjunct to use with CHX. When combined with CHX, the average MIC with CCCP was slightly higher, but a smaller range of MICs was observed (0.67-8.00 µg/ml) compared to EDTA (1.00-16.00 µg/ml), which shows CCCP had a more uniform effect on the test organisms.

The average MBC using EDTA was a lot higher (11.84 µg/ml) than the MBC when CCCP was used (4.34µg/ml). When combined with CHX, the range of MBCs observed with EDTA (1.00-64.00 µg/ml) was larger than the range of MBCs observed with CCCP (1.00-8.00 µg/ml). This shows that there was a large variation in the effect of CHX when EDTA was used. Out of the strains tested, the *K. pneumoniae* strains were less susceptible to the effects of EDTA and CCCP compared to the *E. coli* ones.

In conclusion, the outer membrane of carbapenem-resistant bacteria as well as efflux pumps have a negative impact on antimicrobial effectiveness. This was demonstrated by the effect seen on CHX activity when a membrane permeabiliser (EDTA) or an efflux pump inhibitor (CCCP) was used.

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## The Knowledge and Perceptions of Undergraduate Pharmacy Students on Drugs in Sport

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Doping and the use of performance-enhancing drugs in sport is a growing problem.<sup>1</sup> If appropriately trained, pharmacists can play an important role in doping prevention as well as educating athletes and the general public to make informed decisions about their health.<sup>2</sup> However, it is unclear how many pharmacy programmes include sports pharmacy in their curriculum. The aim of this study was to evaluate the level of knowledge



pharmacy students have around the use of drugs in sport. It also intended to gain an insight into knowledge gaps across a breadth of students, and question if the current teaching is adequate.

The study was conducted through an online questionnaire. This was adapted from a questionnaire created for a study carried out in Qatar.<sup>3</sup> Ethical approval was granted. The questionnaire was circulated by email to four universities. Data had previously been obtained from two further institutions, but had not yet been compared. Quantitative data was entered into SPSS ® v23 and analysed using the Pearson coefficient.

A total of 531 students participated, from six universities. The basic level of knowledge across all students was similar. Participating in sport did not have an effect on knowledge, however year of study did. Ninety-five percent of students believed that sports pharmacy should be part of the MPharm curriculum. Healthcare professionals were thought to be the most reliable source of information to obtain safe and effective advice about drugs in sport. However, there were clear errors in knowledge, predominately around the prohibited status of some substances.

Students expressed a strong desire to learn more about sports pharmacy and participate in doping prevention. However, there are evident gaps in knowledge that need to be addressed before they can provide safe and legal advice to athletes and the public. Further sports pharmacy education is recommended in the MPharm curriculum.

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## Molecular cross-talk between coagulation factor and complement C3

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The coagulation and complement (innate immunity) systems are essential for haemostasis and immune defence, and are activated simultaneously following injury.<sup>1</sup> In disease, where severe trauma and infection has occurred,<sup>2</sup> the activation of these two systems can lead to excessive amplification of the clotting and inflammatory response, causing damage to the host.<sup>1</sup> In this study, we hypothesised that coagulation factor will bind and cleave the initiator of the complement alternative pathway, complement C3.

Indirect and sandwich ligand-binding ELISAs were developed to determine coagulation factor binding to C3, in comparison to thrombin. Gel electrophoresis was used to test the cleavage of C3 by activated coagulation factor. Cleavage fragments were visualised with Coomassie staining and quantified by densitometry.

ELISA studies show that coagulation factor can bind to both adsorbed and antibody-captured complement C3 in a concentration-dependent manner. Activated coagulation factor cleaved complement C3 to C3b as seen by the appearance and increase in band intensity of the  $\alpha'$ -chain of C3b. Under these conditions, no further cleavage of C3b to iC3b was detected.

Coagulation factors ability to bind and cleave complement C3 to C3b suggests that coagulation factor may have complement-stimulatory activity in-vivo. This may contribute to complement alternative pathway amplification, and an increased inflammatory response contributing to the pathophysiology in disease.

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## Evaluation of a community pharmacy-based asthma care plan

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Asthma is a widely prevalent chronic respiratory condition, with over 5.4 million people diagnosed in the UK.<sup>1</sup> Asthma control can be achieved through the use of inhalation treatment, although medication is commonly misused by the patient population.<sup>1,2</sup> The three possible causes of poor asthma control are ineffective medications, lack of patient adherence, or inadequate inhaler technique. This study aims to evaluate the effectiveness of asthma care plans in the pharmacy setting by examining factors such as patient asthma control and inhaler technique.

A total of 61 patients were recruited to the study by convenience sampling across four pharmacies in South Wales. Patients completed an ACT (Asthma Control Test) questionnaire, demonstrated inhaler technique using an AIMS (Aerosolised Inhalation Monitor) Vitalograph device, and answered questions about their asthma management. Ethical approval was granted by Cardiff School of Pharmacy and Pharmaceuticals Sciences' Research Ethics Committee.

Overall asthma control was found to be poor, with 55% (n=33) of patients presenting with "uncontrolled" symptoms. Hospitalisation or use of oral steroids had occurred in 31.3% (n=19) of patients within the last year, further highlighting the scale of inadequate control. Following AIMS testing, MDI technique was revealed to be significantly worse than DPI devices (60.7% versus 20.0% failing, respectively,  $p < 0.05$ ). Asthma control was significantly worse in ex-smokers than non-smoking patients sampled ( $p < 0.05$ ). No significant improvement was seen in returning patient populations from the previous year of study.

Current interventions made by HCPs (Healthcare Practitioners) are failing to adequately address poor asthma control across the population. Improvements to current asthma care plans in the community setting are needed, with focus on re-education and evaluation of adherence and inhaler technique.

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## Evaluation of the Community Pharmacy Flu Vaccination Service in Wales over the last five years (2012-2017)

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Influenza ("Flu") is a contagious disease most prominent between October-April ("a Flu season"). Approximately 600 deaths a year in the United Kingdom result from Flu.<sup>1</sup> Annual vaccination is recommended as the most effective prevention strategy with a target of 75% vaccination uptake in specific patient populations, such as those at risk of contracting severe disease, including sufferers of chronic conditions and the elderly.<sup>2</sup> Before 2012, immunisation in Wales was conducted in General Practice (GP) but vaccination rates remained below the 75% target.<sup>3</sup> As a result, National Health Service (NHS) Wales introduced the NHS Seasonal Flu Vaccination Programme ("the Flu programme") to Community Pharmacy.<sup>4</sup> The aim of this study was to evaluate the Flu programme between 2012-2017.

Secondary data was used in the study and was obtained through online patient forms completed at the time of vaccination between 2012-2017. The resulting data included a range of variables from pharmacy location to patient gender. The data was coded into a quantitative form and analysed through descriptive tests using SPSS Statistics and Microsoft Excel. Ethical approval was not required for anonymised secondary data.

A resulting 67,714 vaccinations were administered over the study period. Overall, vaccination figures increased each season with 1,568 patients immunised in 2012/13 up to 26,889 in 2016/17. In addition, higher proportions of patients are benefitting from the service in the early months (September-November). Most vaccinations (78.4%) were performed in multiple pharmacies (six or more branches). Most patients were female (57.5%) and the largest eligible group using the service was those aged 65 and over with 37,885 (56.0%) vaccinations.

Since its introduction in 2012, the Flu programme is becoming a more prominent service with increasing numbers of vaccinations performed annually. In addition, more patients are receiving immunisation during the earlier months of the Flu season, resulting in protection before Flu begins to circulate.

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## Investigating the antibacterial effect of Namibian honey samples against clinically significant bacterial pathogens, including antibiotic resistant methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumoniae*

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The aim of this project was to investigate the antimicrobial activity of Namibian honey samples against pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug resistant *Klebsiella pneumoniae*, then isolate responsible compounds. Honey is historically renowned for its medical significance.<sup>1</sup> Antibiotic resistance is a significant global threat, with statistics showing that in 2016, some countries had over 25% MRSA resistant strains and one third of *K. pneumoniae* isolates were resistant to at least one of these antibiotic groups: cephalosporins, fluoroquinolones and aminoglycosides.<sup>2</sup> Therefore it's a logical decision to explore honey further.

An agar disc diffusion assay<sup>3</sup> determined the antimicrobial activity of Namibian honey against MRSA, methicillin-sensitive *Staphylococcus aureus* (MSSA), *K. pneumoniae* and *Escherichia coli*. A rotary evaporator for solvent extraction, used methanol, ethyl acetate and hexane to separate compounds differing in polarity. Individual compounds were separated using thin layer chromatography (TLC), before a bacterial overlay assay<sup>4</sup> identified antimicrobial activity. A final agar disc diffusion assay with the methanol extracts in the presence and absence of catalase determined the presence of hydrogen peroxide.

Two out of three Namibian honey samples showed antibacterial activity against target bacterial strains: Windhoek honey showed greatest activity against Gram-positive bacteria; Neudamm honey against Gram-negative. Methanol extracts produced the greatest mass, indicating most honey compounds were polar molecules. Despite showing no results on the bacterial overlay assay, methanol extracts demonstrated antibacterial activity in the agar diffusion assay, both before and after treatment with catalase, suggesting that activity was not due to hydrogen peroxide's presence. Therefore the majority of antimicrobial properties were due to either a synergistic relationship between polar compounds, sugar content or an unknown non-peroxide molecule.

Namibian honey samples demonstrated inhibitory activity against MRSA, MSSA, *K. pneumoniae* and *E. coli*. Further exploration may establish those exact compounds responsible as promising factors in the fight against global antimicrobial resistance.

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## How can technology overcome the barriers faced by community pharmacists to fulfil their future role?

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It is evident that community pharmacists' clinical knowledge is being utilised in the delivery of a variety of patient centred services and the role of the pharmacist is particularly expanding within the primary care sector.<sup>1,2</sup> While the pharmacy profession is evolving, the community pharmacy environment has been slow to catch up as the focus remains on the dispense and supply process.<sup>3</sup> Technology has been shown to improve efficiency and safety notably through improvements in dispensing and management of medicines.<sup>4</sup> This study identifies the perceptions of community pharmacists on the future of their profession and identifies the technological requirements needed to support and enhance these roles.

Two focus groups (with eleven participants) were conducted via a semi-structured method, which were then transcribed ad verbatim. Thematic analysis with an inductive approach was undertaken and three key themes identified.

The future of community pharmacy, barriers to the future role and future technological requirements were the three themes identified. Moving away from dispensing and towards a more clinical role was identified as an aspiration for the future. The inability to access patient records, time spent accuracy checking, and the recent funding cuts were the main barriers discussed. Technological advancements to improve supply, the patient's experience, integration and documentation are necessary to support pharmacists in the future. Access to electronic health records was also identified as a future requirement to ensure patient safety.

Technological advancements should aim to overcome the barriers identified and tackle the current limitations of technology to ensure that the future role of community pharmacists can be achieved without compromise. This study's results can be used by technology developers to improve current practice and enable community pharmacists to utilise their skills, which in turn will result in a more efficient NHS system.

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## Design and synthesis of novel CYP51 inhibitors as therapeutics for candida infections

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In fungi, the CYP51 enzyme is essential in producing the cell membrane component ergosterol. Inhibition of CYP51 with azole drugs leads to loss of ergosterol production, and the production of sterol by-products toxic to the fungi.<sup>1</sup> However, antifungal resistance to licensed azoles is emerging.<sup>2</sup> A new antifungal azole may help combat this, and a lead imidazole compound had been successfully developed by Dr. Claire Simons. The imidazole lead had been shown to be an effective inhibitor of *Candida albicans*, the most common pathogenic human fungi.<sup>3</sup> The aim of this project was to further develop this lead compound.

To investigate potential structural improvements, nine novel inhibitor candidates based on the lead were docked into CYP51's protein structure,<sup>3</sup> using the program Molecular Operating Environment (MOE). Following this, the synthesis of three of these compounds was attempted via a multi-step synthesis.

The drug candidates bound well with CYP51 in MOE, and whilst the synthesis steps were completed, isolation of the final target compounds was not achieved. Fortunately, colleagues in the research group were able to synthesise three of the compounds, which were sent to be tested against *C. albicans* at Swansea University.

One of these, **5d**, contained a new dichlorophenyl moiety and showed considerably improved activity on the lead, reducing the minimum inhibitory concentration required from 4-8 µg/mL to 1 µg/mL.

Compound **5d** warrants further development. Future steps could include extension of the compound's structure to fill unoccupied space in the CYP51 enzyme binding site, or replacing the imidazole with a triazole or tetrazole substituent. With continued development a new clinical treatment for *Candida* infection could be obtained.

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## A study of the physical stability of neonatal parenteral nutrition lipid emulsions in multilayer mini-bags and plastic syringes.

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In 2014 there were three deaths from *Bacillus cereus* septicaemia attributed to contaminated neonatal parenteral nutrition (PN) manufactured by ITH Pharma, with a further 20 infants becoming seriously ill.<sup>1</sup> Cardiff and Vale University Health Board currently test neonatal PN formulations for bacterial contamination simultaneously with delivery to patients. To improve patient safety the Board wishes to move to testing prior to delivery of the PN to the clinical area, requiring formulations to have a longer shelf life than 7 days.

The aim of this study was to assess the physical stability of two different neonatal formulations, containing two lipid emulsions (plus associated vitamin components), when stored in 50ml syringes and 300ml mini-bags over variety of time periods and storage temperatures. The aim of the second section of the study was to assess whether a "pooling" method of manufacture had any effect on the physical stability of the lipid emulsions contained within the formulations. Visual inspection, pH measurement, laser diffraction and microscopic globule size determination were used to assess the stability of each test PN formulation.

Visual changes seen, were easily reversed by shaking therefore were not associated with instability, whilst the pH consistently fell with time but on no occasion did it drop below pH5.<sup>2</sup> Laser diffraction analysis, identified no significant changes in the D[4,3] value apart from the samples based on Intralipid® as the lipid emulsion, when the PN was stored at higher temperatures. There was no significant difference in the largest globule size or the number of globules greater than 10µm. Visual changes, pH values and laser diffraction showed similar results in section two of the study with no clear advantage from the "pooled" method of manufacture being demonstrated.

This study suggests that neonatal PN formulations can have a shelf life longer than 7 days, but was unable to demonstrate a clear difference between the individual lipid emulsion products and/or storage/delivery conditions. Further work is necessary to confirm the stability of the lipid emulsions with these PN neonatal formulations and would be better carried out using 50ml as opposed to 300ml mini-bags.

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## Development of an aptasensor for the detection of procalcitonin

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Sepsis is a life-threatening illness, killing up to 64000 people in the UK each year.<sup>(1)</sup> Accurate diagnosis and timely initiation of treatment are vital to improve a patient's chance of recovery. Currently, there is no gold standard test to diagnose sepsis. A number of potential biomarkers that could aid in diagnosis have been

investigated. Procalcitonin (PCT) is one such example.<sup>(2)</sup> This research aims to develop a biosensor for PCT using aptamers and electrochemical impedance spectroscopy (EIS) which is able to detect clinically relevant PCT levels.

Different self-assembled monolayers (SAM) containing anti-PCT aptamer were modified onto a gold surface electrode by thiol interaction.<sup>(3)</sup> Cyclic voltammetry and EIS were used to characterise the different SAMs on the surface and evaluate binding performance when challenged with increasing PCT concentrations. EIS measured the change in impedance, plotted as Nyquist plots. The equivalent circuit of the EIS Nyquist plots was used to calculate Rct values and the change in Rct after PCT binding, which was used to evaluate the SAM performance as an aptasensor.

Three different approaches were investigated. SAM of aptamer only and co-immobilization of aptamer with different alkanethiol 'spacers' did not produce consistent results. The third approach was more successful, producing an aptasensor with 0.5  $\mu$ M aptamer which was later backfilled with 0.5  $\mu$ M MCH. This sensor demonstrated a concentration dependent response between 10 and 100 pg/ml, with an apparent Kd of 3.26 fM. The limit of detection obtained suggests that the sensor could find utility in clinical testing.

The final aptasensor developed shows promise in PCT detection, however further research will be needed to refine sensitivity for sepsis diagnosis. High PCT levels may be indicative of other inflammatory conditions and thus cannot be used alone for sepsis diagnosis.<sup>(2)</sup> A microarray biosensor design capable of detecting multiple biomarkers should be investigated going forward.

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## Mechanisms of cross-talk between coagulation factor and complement component

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Complement and coagulation cascades are activated simultaneously by common stimuli, to fight invading pathogens and limit bleeding.<sup>1,2</sup> Preliminary data showed a potential binding interaction of coagulation factor with complement component. This work aimed to confirm binding and begin functional characterisation of the coagulation factor-complement component interaction and its potential effects on complement activity, including assessing for complement component cleavage and determining effects of coagulation factor on complement component activity.

An enzyme-linked immunosorbent assay (ELISA) was developed to confirm coagulation factor- complement component binding, detect coagulation factor- complement component complexes in plasma samples and to begin characterisation of binding domains. Enzymatic cleavage assays, monitored by gel electrophoresis, were used to determine if coagulation factor cleaves complement component and complement component activity was assessed  $\pm$  coagulation factor. Protein was visualised using Coomassie stain and band intensity analysis carried out by densitometry (ImageJ).

ELISA techniques confirmed that coagulation factor specifically binds complement component. The developed binding assay detects coagulation factor- complement component complexes in plasma. No cleavage of complement component was detected at physiologically relevant concentrations of coagulation factor.

The coagulation factor- complement component binding interaction has a moderate effect on complement component activity. The herein developed binding assays can be used to develop a new diagnostic tool for detecting complement component -coagulation factor complexes in patient plasma samples. Complement component may also influence coagulation factor's function, which requires further characterisation.

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## To develop a rapid diagnostic capable of determining the antibiotic sensitivity of *Staphylococcus aureus*.

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Methicillin resistant *Staphylococcus aureus* (MRSA), caused by the presence of the *MecA* gene<sup>1</sup>, is a threat to human health as patients with infections caused by MRSA are an estimated 64% more likely to die from their infection compared to those caused by methicillin sensitive *Staphylococcus aureus* (MSSA).<sup>2</sup> Therefore rapid detection of the causative bacterium and subsequent informed antibiotic prescribing is imperative to ensure effective treatment. Despite this, there is currently no single test to guarantee reliable identification of MRSA meaning detection of the bacterium can often take several days.<sup>3</sup> The aim of this study is to aid in the development of a rapid nucleic acid hybridisation assay in the hope to reduce MRSA identification times whilst increasing the reliability of diagnoses.

A selection of clinically suspected MRSA isolates and *S. aureus* control strains were phenotypically characterised using Gram-stain, coagulase test and their susceptibility to oxacillin. Isolates were then further classified using our diagnostic *MecA* and *S. aureus* DNA probes and DNA extracted from each isolate via a commercial extraction kit and via microwaves.

Out of a total of 28 *S. aureus* isolates, 22 were phenotypically identified as MRSA with the remaining six isolates classified as MSSA. It was found that, when using DNA extracted via a DNA extraction kit, 70% (n=10) of clinical isolates were correctly identified using the *MecA* probes and 79.1% (n=24) of clinical isolates correctly identified using the *S. aureus* probes. When using DNA extracted via microwaving, both pairs of DNA probes detected all clinical isolates (n=5).

Initial data suggests extracting DNA via microwaves produces more reliable results and lends itself to the development of a rapid diagnostic assay as DNA can be extracted within a matter of seconds. Despite this, repeat experiments using larger sample sizes and a wider range of *Staphylococci* species is required in order to determine the robustness of the assay and the selectivity of the probes.

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## The role of simvastatin in targeting FAK, and related proteins in the reduction/inhibition of migration seen in triple negative breast cancer

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Triple negative breast cancer (TNBC) is an aggressive subtype with a poor outcome. Successful treatment of TNBC is further confounded by the lack of therapeutic targets (oestrogen and progesterone receptors and HER2 receptor). Several preclinical and epidemiological studies have further supported the claim that statins could be of benefit in TNBC. Most cancer related deaths result from metastasis and there is and overexpression of FAK related to TNBC cells. This study seeks to find the role simvastatin may have in inhibiting migratory cells like/associated with focal adhesion kinase (FAK). Unravelling possible specific treatment to this this cancer subtype, and subsequently be used to better prognosis of patients.

MDA231 cells were cultured and lysed for use in methodology processes. These included western blotting, used to distinguish protein levels in treated and non-treated cancer cells. This was done to understand the possible pathways linked with migration. Migration assay was also carried out to confirm inhibition of migration over 24 hours. Finally, immunofluorescence was conducted to view the subcellular localisation of specific proteins.

Simvastatin showed specific targeting of phosphorylated FAK. An important protein in migration and downward cell signalling. GTPase proteins associated with FAK were also studied. Bearing results of increased increase expression of RhoA and decreased expression of Rac1 in treated cell lines.

This study reports a relationship between simvastatin's use, and inhibition of migration seen in triple negative breast cancer cells. This could potentially be used as an adjunctive treatment with chemotherapy in the future.

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## The Knowledge and Perceptions of Pharmacy Students on Drugs in Sport

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The issue of drug doping is still relevant at all levels of sport<sup>1</sup>, reflecting an emerging need for pharmacists who are well educated in sports pharmacy to provide support and advice regarding the use of drugs in sport.<sup>2</sup> This study aims to compare the knowledge and perceptions of students across several pharmacy schools in order to determine the current level of sport pharmacy education in the UK.

The study consists of a questionnaire which has been adapted from one previously conducted in Qatar.<sup>3</sup> The questionnaire was circulated to: Cardiff University, University of Birmingham, University of Reading, Kingston University London, Aston University and University of Bath. Data generated was mostly quantitative and was analysed using SPSS v23. The Pearson correlation coefficient was used to analyse respondents' knowledge, at the significance levels  $p < 0.01$  and  $p < 0.05$ .

Across the six schools, there was a total of 531 respondents. Results showed a statistically significant relationship between year of study and correctly identifying the prohibited status of amphetamines, anabolic steroids and codeine at the level  $p < 0.01$ . 92% of respondents correctly identified the prohibited status of anabolic steroids but only 12% correctly identified the status of insulin. 97% of respondents believed that pharmacists should be used as healthcare professionals to provide advice to athletes and 95% stated they thought sports pharmacy should be included in their curriculum, as either a core or elective module. Results showed disparity in the quantity of teaching on sports pharmacy that different schools provide.

Knowledge of students is good but gaps in knowledge need to be addressed. In order to be pharmacists who are better equipped for the effective administration of advice to athletes within local communities, clearer guidelines which enable the provision of quality teaching on drugs in sport should be employed.

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## Evaluation of the DMR service from April 2013- August 2017

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The Discharge medicines review (DMR) service was developed and implemented in Wales by the Welsh Government in October 2011.<sup>1</sup> The initiative was designed to improve care transitions. The service was evaluated in 2014.<sup>2</sup> However, since then there have been no evaluations of the service. The aim of this project was to evaluate the DMR service from April 2013 – August 2017.



Five researchers used the Statistical Package for the social sciences (SPSS) ® software to combine existing DMR data. Researchers conducted secondary analysis of the quantitative data individually using SPSS. Spearman and Pearson correlations, post hoc tests (ANOVA), and the Mann Whitney test were used to statistically analyse data. Ethics approval was not required.<sup>3</sup>

Across all Wales, the number of DMRs conducted significantly increased over time ( $p=0.00$ ). A total of 37425 DMR claims were submitted. Cwm Taf Health Board had a significantly greater number of claims per 100000 of the population ( $p=0.00$ ). It could be suggested that this was influenced by the implementation of a new information technology scheme called Choose Pharmacy, which changed the process of the DMR service. This scheme was introduced because of suggestions made in the 2014 evaluation.<sup>2</sup> Of the accredited pharmacies that can provide the DMR service, only 25% participation was observed monthly. As each pharmacy can undertake 140 DMR's per year, an average of 0.71% of these potential DMRs were provided across all Wales. The discrepancy rate is still 1 per DMR, as it was in the 2014 evaluation.<sup>2</sup>

The results demonstrate that there is still underutilisation of the service. Further studies are required to explore the reasons for this as well as the benefits and limitations of the service to inform future improvement.

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## The effect of Welsh honey and other natural products on bacterial biofilms

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In the United States, it has been estimated that 65% of all human infectious diseases can be attributed to biofilm-forming bacteria.<sup>1</sup> These 'medical biofilms' are commonly found in catheters and ventilator tubes. They are also common among patients with cystic fibrosis.<sup>2</sup> Bacteria form biofilms by secreting and growing a polysubstance matrix, this affords the bacteria protection from the environment and makes them extremely resistant to antibiotics. The aims of this study were to determine the susceptibility of *Pseudomonas aeruginosa* (NCTC 10662) and *Klebsiella pneumoniae* (clinical isolate) to four natural products: Welsh Redwood honey, manuka honey, black tea and hops and to perform biofilm inhibition and elimination assays against these bacteria.

To test the susceptibility of the bacteria, a zone of inhibition was performed with the natural products. To calculate the phenol coefficient, phenol (1-7% w/v) was also tested. To test the natural products ability to inhibit and eliminate biofilms, bacteria were grown in a 96-well titre. Biofilm was quantified by dyeing it and analysing the optical density at 570nm.

Bacteria were susceptible to Redwood and manuka honey. Redwood and manuka honey appear to inhibit biofilm formation. Redwood honey at high concentrations eliminates established biofilms. Black tea inhibited *K. pneumoniae* biofilm formation at concentrations of 12.5% v/v and higher, but was unable to eliminate biofilm. The bacteria appear to be unaffected by the presence of hops.

Redwood honey has potency comparable to manuka honey. Black tea shows antibiofilm properties. More optimised biofilm assays need to be performed to verify the results. More research needs to be performed on Redwood honey and black tea to deduce the active components and their mechanism of action.

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## Development of Aspartyl-AMP mimics to treat Multi-Drug Resistant (MDR) *Pseudomonas aeruginosa*

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The incidence of MDR *P. aeruginosa* has been increasing since 2003, and the World Health Organisation has marked the development of new antibiotic to treat it as “critical” in a recent report.<sup>1,2</sup> Infections of *P. aeruginosa* increase mortality and morbidity, and are common in very vulnerable groups.<sup>3</sup> An infection can lead to bacteraemia, which is normally secondary to an untreated infection, making the increase in the incidence of MDR resistance a major health concern.<sup>4</sup> In this project, the aim was to design and develop two or more novel molecules, which are mimics of aspartyl-AMP (Asp-AMP) and would have the potential of inhibiting aspartyl tRNA synthetase (AspRS) in *P. aeruginosa*.

The molecules were designed based on previous research carried out at Cardiff University. Molecular modelling was performed to evaluate the binding of the designed molecules with the target. Chemical synthesis consisted of four steps which were based on previous research found in the literature.

Four mimics of Asp-AMP were designed, but only one was successfully produced which was at a low quantity and not up to an acceptable standard to be tested. However, a synthetic route was derived. Furthermore, the molecule containing the chloro-substituent was found to be the one with the greatest potential of inhibiting AspRS during molecular modelling.

The derived synthetic route will enable the future development of the designed analogues. Suggestions are provided on how the purity and yield of the product could be improved. The impact of our research is highly dependent on the results that will be received from biological assays of molecules that will be produced in the future using the derived synthetic route.

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## Evaluation of Discharge Medication Reviews in 2017

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Discharge Medication Reviews (DMRs) are a service provided by community pharmacists in Wales to ensure patients' medications are accurately prescribed in the community after discharge from hospital. Medicines are often altered during hospital admission and this information is not always effectively communicated to the patient's GP or pharmacist.<sup>1-3</sup> DMRs identify and resolve discrepancies. They consist of two-parts; a pharmacist's review of the discharge information compared against what has been prescribed by the GP, and a consultation with the patient to advise them about their medication<sup>3</sup>. The aim of the study was to evaluate the DMR service since 2013, and identify trends year on year.

A descriptive and statistical analysis was performed using Statistical Package for Social Sciences (SPSS) with secondary data provided by NHS Shared Services.

Results show the number of DMRs completed has remained constant or slightly increased over the last four years, with a mean of around 8,325 per year. When adjusted for population, Cwm Taf carried out the most DMRs and Powys the fewest. Consistently around 700 contractors are accredited to provide the service each year, but only 200 actively participate. Hospitals account for over 50% of referrals to the service and the main reasons for undertaking DMRs were for medicines changed during admission or polypharmacy. A third of consultations were conducted over the telephone with the patient. At least one discrepancy was found on average per DMR; a figure which remained constant over the study period. However, 10-20% of DMRs were not completed and at least 25% of discrepancies remained unresolved.

DMRs can undoubtedly make a worthwhile contribution to patient safety by identifying discrepancies. However, more needs to be done to ensure their resolution. There is also vast underutilisation of the service which, if appropriately optimised, could prevent serious harm from medication. The Welsh government should commission further research into the barriers for conducting DMR, adequately resource and promote the service and introduce outcome-based payments for contractors to address these issues.

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## **‘Welsh language in pharmacy practice: confidence of pharmacists and pre-registration pharmacists with formal Welsh language undergraduate tuition’**

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Approximately 19% of the populations in Wales are fluent Welsh speakers.<sup>1</sup> In 1993, the Welsh Language act enabled both the English and Welsh language to have an equal status in Wales within the public sector.<sup>2</sup> The Welsh Language measure, introduced in 2011, gave the Welsh language an official status in Wales where public bodies had to comply with legal standards to provide a bilingual service.<sup>3</sup> ‘More than just words’ is a framework which strengthens the use of Welsh language within healthcare followed by the ‘Active offer’ which ensures that Welsh speaking patients care is provided through the medium of Welsh without them having to request it.<sup>4</sup> As a result of the increase in Welsh language services, Cardiff School of Pharmacy introduced Welsh language tuition within their undergraduate course in 2012. The aim of this project is to investigate how attending Welsh language tuition has affected the confidence of Pharmacists and pre registration Pharmacists in communicating in Welsh to patients.

Ethics approval was obtained, qualitative research was carried out after semi structured interviews were undertaken with five participants. Consent was sought and interviews were audio recorded and transcribed verbatim and thematically analysed. Bilingual topic guides, consent forms and participant information sheets were given to participants prior to interviews.

Study results showed that all five participants believed that Welsh language tuition provided by the undergraduate course increased their confidence in communicating in Welsh to patients. However, there is room for improvement in their awareness of Welsh language initiatives, availability of bilingual resources and in improving their confidence in initiating Welsh consultations. As a result, participants would like to see more Welsh language consultation training being offered during their pre-registration course and greater access to bilingual resources. This will help provide them with the tools to meet the needs of Welsh-speaking patients and improve and strengthen patient care.

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## **Analysis of the major constituents of UK-grown *Artemisia annua* for medicinal and commercial significance.**

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Artemisinin forms the backbone of malarial treatment, particularly in South-East Asia<sup>1</sup>, but due to its structural complexity, cultivation of the plant *Artemisia annua* is the only viable source.<sup>2</sup> While it grows freely in many

parts of the world<sup>3</sup>, this project investigated if UK grown *A. annua* could yield artemisinin and other compounds in sufficient quantity to be of pharmaceutical significance.

Leaves, stems and flowers were all investigated. Different solvents and extraction methods were used to obtain extracts which were separated using preparative thin layer chromatography. The project was split; one student searched for artemisinin, the other student investigated significant bands for a high yielding constituent (bands yielding complex mixtures were abandoned). Sephadex LH-20 was used with little success as a scale up method. Compounds were characterised by ESI-MS. Standards were not used anywhere in the project due to costs. Extracts and fractions were tested against both gram-positive and gram-negative bacteria.

Artemisinin was found at a maximum yield of 0.28% of the dry weight of *A. annua* leaves but this was not significantly viable. The compounds daphnoretin and isoalantolactone were found though these have not been found in this species of *Artemisia* previously.<sup>4</sup> Two compounds showed activity against *MRSA*, these were thought to be parthenolide (not previously found in *A. annua*<sup>4</sup>) and one or both of the compounds within the isomeric mixture of casticin and chrysosplenetin.

The antibacterial activity of these compounds should be further investigated as novel antibiotics. However, as only trace amounts of artemisinin were found in the UK-grown *A. annua* it would not be feasible to grow this plant on a large scale in Wales. It is unclear what factors may have affected the level of artemisinin produced. This could be an area of future research, though a repeat of this project should use plants grown in different parts of the UK and under different conditions e.g. outdoor versus greenhouse grown.

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## Improving the health of community pharmacy influences on pharmacists' uptake to new technology

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The NHS is promoting the use of technology to improve both patient care and enhance the roles of healthcare professionals.<sup>1</sup> The role of community pharmacists is also evolving to develop more clinical roles, but new technologies are required to aid this advancement.<sup>2</sup> As new technologies are produced it is important to know what factors influence the pharmacist to uptake new technology, but there is currently no literature that describes these factors. This research aimed to identify how pharmacists are influenced to uptake new technology. The objectives were to use a literature review to develop a topic guide to for discussions within focus groups to discover the main themes to shape a future survey. This information will influence the design and marketing strategies of technology companies.

The literature review used to develop the topic guide was completed using keywords in a Google Scholar search and by searching Pharmaceutical Journals. Two focus groups were carried out and recordings were transcribed and analysed to extract the main themes and associated subthemes. These were used to construct a survey questionnaire.

The main themes emerging from this research were that pharmacists wanted clarity on: (a) ways of finding out about new technology; (b) identification of the benefits of new technologies; (c) features of the PMR system and (d) how company policy affected introduction of new technologies. Subthemes of issues emerged that were related to each theme.

The findings identified what factors were important to influence a pharmacists' decision to uptake new technology and these formed the basis of the survey questionnaire. The major finding was the importance of company policy, which showed that independent pharmacists make their own decisions to uptake technology, but head office would need to be targeted to influence multiples to uptake new technology.

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## **Evaluation of a community pharmacy-based asthma care plan**

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Asthma is an incurable inflammatory condition of the airways adversely affecting patient quality of life if not treated with medication.<sup>1</sup> Medication is usually inhaled using devices such as metered dose inhalers (MDI) and dry powder inhalers (DPI). 71% of asthmatics are “poor users” of inhalers, which prevents treatment reaching the target site in the lungs and leaving them more liable to exacerbations requiring oral steroids or hospital treatment.<sup>2</sup> The aims of this study were to evaluate inhaler technique and asthma control and observe whether improvement is shown a year following an intervention.

61 participants were recruited from Cardiff University and four community pharmacies. Participants completed an Asthma Control Test (ACT) and a questionnaire, and the Vitalograph Aerosol Inhalation Monitor (AIM) was used to measure inhaler technique. Data analysis was completed on SPSS using Mann-Whitney U and Kruskal-Wallis supported by Dunn’s *post hoc* test. Returning participants’ results were analysed using a related samples Wilcoxon-signed-rank test. P values <0.05 are considered significant. Ethical approval was obtained.

Results showed that, regardless of which health care professional instructed them, inhaler technique was generally poor, with worse technique in MDIs than DPIs (p=0.012). According to ACTs, 50% of participants had uncontrolled asthma. A third of participants have had exacerbations, 75% of which still had uncontrolled asthma. There was no correlation between uncontrolled asthma and poor inhaler technique, potentially due to non-adherence which was not evaluated in this study. Participants returning from last year’s study (n=8) did not show improvement in asthma control or inhaler technique.

Overall, both inhaler technique and asthma control were poor and need improvement. Only 5% (n=3) of participants were shown technique by pharmacists but there is evidence of technique and control improvement when patients have a review with a pharmacist.<sup>3</sup> Tailored education should be provided to patients more frequently than annually as this does not appear to be frequent enough.<sup>4</sup>

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## **The views of Welsh speaking pharmacists who have not received formal Welsh undergraduate teaching; use of and confidence in using the Welsh language in professional practice.**

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The linguistic needs of patients are often overlooked in health and social care, with a lack of language provision a factor in less favourable patient outcomes.<sup>1</sup> In 2012, Welsh medium undergraduate tuition was introduced in the School of Pharmacy at Cardiff University. This followed the introduction of frameworks such as ‘More than just words...’, to improve Welsh language provision in health and social care.<sup>2</sup> The aim of this study was to explore the views of Welsh speaking pharmacists that had not received any undergraduate teaching in Welsh, on their confidence in using the Welsh language in practice.

Qualitative research in the form of semi-structured, audio recorded interviews (n=5) was conducted. Interviews were transcribed verbatim, coded manually and analysed thematically. All materials used with participants was provided bilingually and ethically approved before use.

Four major themes were identified: Pharmacists' perceptions of advantages/disadvantages of Welsh language provision for patients, Barriers to Welsh language provision, Confidence in using the Welsh language and Increasing future Welsh language provision. Participants showed an overall lack of confidence in using the Welsh language in practice. Geographical differences in participants' confidence and demand for Welsh language services was observed. Participants were confident in the use of the Welsh language socially, with some describing the challenges in using the Welsh language professionally due their lack of knowledge of Welsh language terminology.

Participants highlighted several regions for change in Welsh language provision, both at undergraduate and postgraduate levels, that would be beneficial in increasing their confidence in providing Welsh language services. Participants also highlighted the need for more Welsh language resources, in the facilitation of future Welsh language services.

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## The Synthesis of Double Branched Deshydroxy Androgen Receptor Antagonists for Potential Prostate Cancer Treatments

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Prostate cancer is the second highest cause of cancer affecting men across the globe, accounting for a fifth of all cancer related deaths in men.<sup>1</sup> The androgen receptor (AR) promotes the growth of prostate cancer cells providing an ideal target for potential therapies.<sup>2, 3</sup> Existing treatments include the non-steroidal androgen receptor antagonist for which bicalutamide is favoured due to its superior toxicity profile.<sup>4</sup> Unfortunately bicalutamide resistance eventually develops leading to terminal cancer.<sup>2, 3</sup> This project aims to overcome bicalutamide resistance by synthesising a series of novel double branched deshydroxy bicalutamide derivatives bearing bulky substituents.

A homology model of the AR in its antagonistic conformation was created to predict antagonistic activity. The derivatives were synthesised using a simple, three step process. The first step involved the synthesis of an amide intermediate using methacryloyl chloride and 4-nitro-2-(trifluoromethyl)aniline. The amide intermediate was reacted via a Michael addition with a series of thiophenols to produce the sulphide before oxidation to the sulphone.

A double branched derivative of low yield (5 %) was obtained and predicted to have good antagonistic activity by homology modelling. The remaining four sulphides were single branched with variable yield (71-52 %) and were successfully oxidized to produce two pure and two impure sulphones. Oxidation of the double branched compound was unsuccessful.

The pure compounds will be tested in human prostate cancer cell models to evaluate anti-proliferative activity. The synthesis of double branched derivatives was largely unsuccessful, requiring further research to obtain a reliable, high yielding method.

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## An *ex-vivo* investigation into the bladder wall distribution of ketamine using a porcine bladder model

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Ketamine has been used clinically since 1970, with its primary use in anaesthesia.<sup>1</sup> Due to its effects, it has been abused recreationally since 1980.<sup>2</sup> The use within nightclub settings is increasing and it is known as a “date-rape” drug.<sup>3</sup> Following reports of lower urinary tract symptoms in ketamine users in 2007, a condition known as ‘ketamine-induced cystitis’ (KIC) emerged.<sup>4</sup> The aim of this study is to investigate the permeation of ketamine across the bladder wall by determining ketamine concentrations achieved in each bladder wall layer. Concentration-depth profiles will then be constructed.

An *ex-vivo* porcine bladder model was created to replicate normal physiological conditions of the human bladder. The method used builds upon models based on intravesical drug instillation, whilst taking into account the effects of drug excretion and urine dilution. Following each permeation study, three tissue samples each measuring 1.5cm x 1.5cm were taken from each bladder, rapidly frozen and cryosectioned into 50µm sections. Quantification of ketamine was carried out following drug extraction from tissue samples and analysed using high-performance liquid chromatography-ultraviolet detection. Six bladders were used in each experiment (n=6).

The excretion mimicking study involved calculating ketamine concentrations expected inside the bladder and instilling this into the *ex vivo* bladder every 20 minutes. Ketamine permeated across the entire bladder wall, with the highest concentrations observed in the urothelium (167.96 µg/µg). Concentrations of 122.04 µg/µg and 70.46 µg/µg were found in the lamina propria and detrusor respectively. Results showed that permeation across the bladder wall occurs in a dose and time-dependent manner and support Fick’s 1<sup>st</sup> law of diffusion.

As KIC is a potential life-changing and not a well-understood condition, there is the need to raise awareness. This study provides an understanding of ketamine distribution within the bladder, aiding future studies to explore the pathophysiology.

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## Use of a Multi-Asperity Adhesion Model to Determine the Effects of Material Properties and the Influence of Adsorbed Albumin on Bacterial Force of Adhesion.

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Dental plaque, a complex oral microbiome, is the primary causation factor in dental caries.<sup>1</sup> The colonisation of a tooth surface or dental biomaterial by pioneer bacteria species is initiated by the adsorption of salivary proteins on a surface. Surface characteristics and the presence of adsorbed proteins can affect the extent of bacteria adhesion.<sup>2,3</sup> The mechanism of bacteria-material surface and bacteria-adsorbed salivary proteins interaction is not fully understood.<sup>2</sup> Therefore, this study aims to evaluate how material properties affect bacteria adhesion and compare the effects of adsorbed albumin (bovine serum albumin) on material surfaces on bacteria adhesion.

A multi-asperity model based on the JKR theory, designed by Dr Prokopovich and Perni<sup>4,5</sup> was used to 1. Estimate adhesive force between a biomaterial and bacteria and then identify the least fouling biomaterial and 2. Estimate adhesive force of bacteria adhesion in the presence albumin (bovine serum albumin) coated biomaterials. Quantitate data were analysed using SPSS and comparison tests were carried out using Tukey, ANOVA and an independent samples t-test.

In the presence of adsorbed albumin coating on composite resin and titanium, *S. mutans* and *S. salivarius* showed a significant reduction in adhesion force, whereas *S. aureus* and *A. actinomycetemcomitans* showed



an increase in adhesion force when compared to uncoated surface. Results also showed that *S. mutans* was the most adhesive bacteria whilst *S. aureus* was the least adhesive bacteria overall. In general, higher bacteria adhesion were observed on surfaces with high surface energy and low surface elasticity.

In conclusion, Yttria-stabilised Zirconium was the least fouling dental biomaterial. Bacteria 'stickiness' is related to its surface elasticity. Adsorbed albumin reduces surface hydrophobicity which can either cause an increase or decrease in bacteria adhesion force. However, bacteria adhesion is a multi-factorial process; therefore, further studies should be performed in the future.

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## An investigation into the risk factors that may contribute to elderly members of the public having a fall

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Falls amongst the elderly is a common problem resulting in an estimated 230,000 to 460,000 people over the age of 60 falling in Wales each year.<sup>1</sup> Additionally, 30% of people over the age of 65 and 50% of people over the age of 80 fall at least once a year.<sup>2</sup> Such figures have contributed to falls becoming the second leading cause of accidental or unintentional injury deaths worldwide, with the greatest number of fatal falls coming from adults over the age of 65.<sup>3</sup> This study aims to identify associations between a number of pre-determined risk factors and the occurrence of falls within the last 12 months of patients presenting at community pharmacies in Wales.

All 716 community pharmacies in Wales at the time took part in the national falls campaign 'Steady On ... Stay SAFE' from February through to March 2017<sup>4</sup> and were sent 20 questionnaires each for members of the public to complete. Resulting quantitative data was analysed using the chi-square statistical test to compare the pre-determined risk factors. Ethics approval was obtained.

36% of the participants had a fall within the last year, with 80% of those people over the age of 60. Aside from blood pressure pills and eye drops for glaucoma all medications provided a  $p = < 0.001$  showing a statistical difference in the rate of respondents having a fall in the past 12 months. Polypharmacy ( $p = < 0.0001$ ), over the age of 70 ( $p = < 0.0001$ ), gender ( $p = < 0.0001$ ) and 13 of the 14 combinations of the listed drugs/classes of drugs also provided a statistical difference.

Study showed taking medications, combinations of those medications, age and polypharmacy to be the major risk factors contributing to falls. Highlights the need for action through medication reviews and interventions.

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## Exploring the Relationship between STYK1 and the AKT pathway in ER+ breast cancer by monitoring FOXO3A in preclinical models and clinical disease

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Endocrine resistance is a major problem in oestrogen receptor positive (ER+) breast cancer, leading to relapse.

<sup>1</sup> Mechanisms of resistance are poorly understood but researchers in Cardiff University recently found that a novel receptor tyrosine kinase, STYK1, is increased and drives endocrine-resistant cells *in vitro*. They also detected STYK1 in an ER+ clinical breast cancer series, so STYK1 might feasibly contribute to relapse. It is believed STYK1 signals in part via the PI3K/AKT pathway <sup>2</sup> but this mechanism is unexplored in clinical material. The aim of this project was to explore if there was any association between STYK1 and AKT signalling in ER+ breast cancer by monitoring FOXO3a, a transcription factor downstream of the AKT pathway.

FOXO3a is excluded from the nucleus when AKT is active, blocking its tumour suppressor function.<sup>3</sup> An immunohistochemical (IHC) assay was thus optimised that could detect nuclear and cytoplasmic FOXO3a in stored breast cancer sections. The assay was run on sections from the ER+ clinical series (n=55) pre-assayed for STYK1 and endocrine resistant cell pellets and xenografts. H-scoring evaluated nuclear and cytoplasmic staining. Statistical analysis explored relationships between FOXO3a, STYK1 and further biological/clinicopathological parameters.

STYK1 and cytoplasmic FOXO3a were significantly correlated (p=0.01) in the clinical samples. Cytoplasmic FOXO3a also correlated with ERK1/2 (p=0.044, p=0.009) MAPK expression and activity (p<0.001). There was a significant association between nuclear FOXO3a and tumour size (p=0.025). Endocrine resistant pellets and xenografts stained for cytoplasmic FOXO3a and where examined, STYK1.

The optimised IHC assay provides evidence that STYK1 may activate AKT signalling, promoting cytoplasmic localisation of FOXO3a, in ER+ breast cancer and preclinical resistant models. Its further mechanistic findings are supportive of FOXO3a also lying downstream of MAPK. <sup>4</sup> However, the clinicopathological association with nuclear FOXO3a was surprising given its suppressive function, suggesting assay improvements may be needed using increased samples with endocrine outcome data.

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## The Repeat and Managed Repeat Prescription Ordering Service: The Patient's Experience

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With the National Health Service (NHS) under more pressure than ever to deliver services at an acceptable standard with intensifying demand by a population with its life expectancy on the rise, the optimisation of fundamental services is a priority to ensure the implementation of cost-effective processes such as the Repeat Prescription Ordering Service (RPOS) and Managed Repeat Prescription Ordering Service (MRPOS) <sup>1</sup>. The aim of this study is to determine whether these services are user-friendly and easily understood.

Participants were initially recruited via stratified sampling followed by snowball sampling<sup>2</sup> to recruit the remainder. The research was carried out within the Abertawe Bro Morgannwg, Cardiff and Vale and Hywel Dda Health Board regions. Semi-structured interviewing<sup>3</sup>, was used to gather qualitative data on patient views and/or experiences. The interview schedule was designed so that questions were focussed on fundamental aspects that make up the medication ordering services. Data was analysed qualitatively via thematic analysis<sup>4</sup>. Codes were developed after transcription of interviews were complete and these were categorised into themes.

Positive and negative features of the RPOS/MRPOS were successfully identified and possible solutions were suggested by participants to overcome these barriers. Firstly, 30% of participants had the inability to identify who was responsible when something in the process went awry due to miscommunication between the pharmacy and the surgery. A new communication system was suggested by a participant. Secondly, 70% of participants appreciated the convenience of RPOS/MRPOS; particularly those who were housebound and used the MRPOS. Participants mentioned that the pharmacy were accommodating; requesting a prescription from the surgery for a patient going on holiday.

There is not a unanimous opinion that enables a decision to be made concerning the continuation of these services, however, with more research involving a wider population, it is possible that this service will be developed or replaced.

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## Assessing the permeation of propranolol hydrochloride cream for the prospective treatment of infantile haemangiomas

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Infantile haemangiomas (IH) are raised swellings on the skin and affect around 4-5% of children.<sup>1</sup> They usually shrink on their own, however, up to 10% of cases can cause problems and require further treatment.<sup>2</sup> Since it was discovered that oral administration of the drug propranolol was effective in treating IH in 2008, it is now the first line treatment.<sup>3</sup> However, unwanted systemic side effects can be associated with oral propranolol administration. This study aims to determine whether a topical formulation of propranolol hydrochloride (PH) could potentially be used for this condition in order to avoid these unwanted systemic side effects.

Three creams were formulated by adding powdered PH to pre-formed cetrimide creams at the strengths 2%w/w, 5%w/w and 10%w/w (termed 'solid-phase' creams). A further cream was prepared at 2%w/w PH whereby the PH was added to the water phase during formation of the cetrimide cream (termed 'liquid-phase' cream). Franz-diffusion cells were used to test the permeation of the creams permeating through an artificial membrane. Samples were taken from the lower chamber of the diffusion cells at designated time intervals and used to determine the concentration of PH cream that had permeated through the membrane.

The 'solid-phase' 2%w/w was more permeable than the 'liquid-phase' ( $p=0.992$ ), however, all strengths demonstrated a level of drug permeation. Increasing the strengths of the 'solid-phase' creams also increased the permeability, which was demonstrated when comparing the 2%w/w and 10%w/w cream ( $p=0.026$ ).

From this research, it can be concluded that the manufacturing process and the strength had an effect on the permeation of the PH creams. The data from this study provides encouragement that topical formulations may be suitable for IH. However, research needs to now test these formulations on skin tissue to determine the safe and effective therapy for children.

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## The effect of hot and cold drinks on the dissolution of gelatin and hydroxypropyl methylcellulose (HPMC) capsules.

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Oral capsules are a major form of medication delivery and can be manufactured from gelatin, traditionally, or HPMC, in more recent years. Gelatin is soluble in stomach fluids at body temperature but its dissolution can be adversely impacted by storage conditions.<sup>1</sup> HPMC has been shown to be less affected by storage conditions but more susceptible to oxygen entry, therefore less suitable for oxidation prone drugs.<sup>2</sup> Dissolution of capsules releases drug into the stomach for absorption and thus is an important performance consideration. Dissolution machines assess the capsules' ability to release suitable amounts of drug to avoid the need to test in-vivo on a regular basis.<sup>3</sup> The aim of this study is to determine the impact of hot and cold drinks on the dissolution of gelatin and HPMC capsules as this may affect how much drug a patient receives, or at what rate.

Dissolution testing was performed on dye-filled capsules at various temperatures and imaged using a high-speed camera to show the exact beginning of dissolution. Further capsules were filled with propranolol hydrochloride (as model drug) and samples of dissolution medium analysed using UV VIS spectrometry to show the mass of propranolol in solution following capsule dissolution (n=3). A two-way ANOVA with post hoc Tukey's HSD test compared the mass of propranolol in solution between the different temperatures and capsule types.

Imaging showed capsules first break at the shoulders; the weakest point.<sup>4</sup> Gelatin dissolution increased at higher temperatures while HPMC remained relatively consistent. The mass of propranolol released at 24°C was significantly lower (p<0.05) than at higher temperatures. The cumulative mass of drug released over all temperatures from gelatin and HPMC capsules was significantly different but, aside from 24°C, the mass released from each type at the same temperature was not.

In summary, this tentatively suggests that capsules should not be taken with cold drinks as they can decrease drug dissolution, therefore the patient may not receive a full dose.

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## Development of standardised tests for microneedles

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Microneedles are micron-sized needles that facilitate the delivery of macromolecules by penetrating the *stratum corneum*.<sup>1,2</sup> A huge advantage of this approach is that microneedles can increase skin permeability to a drug by up to 10,000 times.<sup>3</sup> However, currently there are no approved regulatory tests for microneedles. Thus, the purpose of this project is to start developing new standardised regulatory tests for 'loss of moisture' and 'dissolution'. The development of such tests will be important when microneedle devices are undergoing clinical trials and when marketing authorisation is required.

Stainless steel microneedles were coated using a formulation of C19-A3, a proinsulin peptide, with an attached fluorophore. The 'loss of moisture' test was conducted by placing either a 0.2µl or 0.6µl drop of formulation on the surface of a microneedle and recording the change of weight with respect to time. The microneedles were coated and the coating was then removed in the dissolution studies, using a novel 3D-printed dissolution apparatus. Quantification was measured via UV-Vis absorbance or fluorescence spectroscopy.

Major findings include consistent and linear loss of moisture for both 0.2µl and 0.6µl formulation volumes at a rate of 1.3  $mg\,s^{-1}$ . There was insignificant weight loss after 90 and 360 seconds for the lesser and greater drop size respectively. The dissolution studies showed acetic acid worked better as a dissolution solvent than PBS.

It was also discovered that conducting a dissolution study in either 200µl or 400µl of solvent or increasing the dissolution time did not affect drug dissolution, as the quantity of peptide analysed was measured to be the same.

There is still a long way to go to create standardised tests that can be recognised and adopted by regulators. This thesis has demonstrated how some parameters for these tests will be easier to harmonise than others.

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## Investigating the behaviour of keratinocytes in a fibroblast co-culture

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Bio printing is an exciting new approach in regenerative medicine and printing an authentic skin substitute could provide many useful applications in the clinic and the laboratory.<sup>1</sup> Keratinocyte and fibroblast cells play important roles within the structure of human skin<sup>2</sup>, therefore their compatibility is integral to constructing a physiological 3D representation of the tissue. Cell function is regulated via paracrine signals from soluble factors that are secreted by each of these cells in a feedback loop.<sup>3</sup> In this study, the cell behaviour in a co-culture of keratinocytes and fibroblasts is explored via the use of fibroblast conditioned medium (CM). Calcium is a major modulator of keratinocyte differentiation<sup>4</sup> and so the effects of fibroblast CM on keratinocyte behaviour will be investigated in both low calcium (LC) and high calcium (HC) conditions.

Fibroblasts (HCA<sub>2</sub>) were grown to 80-90% confluency and fresh medium was conditioned for 24hrs before being collected. LC and HC HaCaT keratinocytes were seeded at a density of 2x10<sup>4</sup> per well and cultured in fibroblast CM or commercially available DMEM as the control, the rate of growth and morphological changes were observed over 6 days.

Addition of conditioned medium significantly slowed the rate of proliferation in both HC and LC keratinocytes. Morphology changes were observed in both cell types; cells became elongated, spindle shaped and much larger in size with visible adherence proteins.

The results suggest that there may be a growth factor or signalling molecule in the conditioned medium which slows proliferation of keratinocytes. This suggests that further work would need to be conducted to optimise conditions for a 3D bio printed model. Further experiments are needed to understand these behavioural changes.

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## Development of methods to characterise hydrogel properties

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The aim of this study was to develop a universal method to characterise at the microscale different hydrogels that are produced from a microfluidic system, to understand the relationship between gelation and changing of parameters in gel production.

Gelled hydrogels were produced in a custom fabricated microfluidic system. The bio-compatibility of hydrogels means they are attractive materials for use in many many bio-engineering applications. For example hydrogels have been used to encapsulate droplet bilayer networks and encapsulate live cells. However, characterisation of the structural and mechanical properties of the gels on the microscale remains challenging.

The progressive development of a feasible method of characterisation begun with a preliminary method design, to measure contact angle in order to quantify the level of gelation. Although only qualitative results were produced, apparatus setup were gradually improved and it led to the discovery of the relationship between integrity of the hydrogel and their compressive properties. Using Xforce force-measurement system, gelation conditions and mechanical properties of the resultant gel were quantified and correlations established.

The novel method to characterising microfluidically generated hydrogels potentially provides a route to in-depth understanding of the dependency of hydrogel properties on their manufacture conditions.

## **Generation of a highly sensitive electrochemical biosensor for the detection of C-Reactive Protein**

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Sepsis occurs when localised infection cannot be terminated by the host's immune system, resulting in the development of widespread inflammation.<sup>1</sup> The condition affects ~123,000 people and is responsible for ~37,000 deaths in England annually.<sup>2</sup> Promptness of sepsis diagnosis is a key factor that influences clinical outcome. C-reactive protein (CRP) is an inflammatory biomarker which has been able to predict organ failure and guide therapy for septic patients.<sup>3</sup> In this study, an electrochemical aptamer-based biosensor for CRP was developed.

Gold working electrodes were sonicated, polished, and electrochemically activated by cyclic voltammetry in 0.5 M sulphuric acid. Following cleaning, anti-CRP aptamers, with or without alkanethiol molecules (6-mercapto-1-hexanol, 4, 4'-dithiodibutyric acid and cysteamine), were chemically immobilised onto the working electrode surface by their reduced sulphide functional group to form a self-assembled monolayer (SAM).<sup>4</sup> CRP was incubated with each SAM for 15 minutes in order to evaluate the binding performance of monolayer. Electrochemical impedance spectroscopy and cyclic voltammetry were performed to monitor monolayer formation on the electrode surface and the effect of CRP binding. The system's selectivity for CRP was challenged with procalcitonin.

After screening several alkanethiol molecules, 6-mercapto-1-hexanol (MCH) was selected as the molecule to combine with the anti-CRP aptamer in ratios. The 1:1 ratio of MCH:aptamer gave a concentration dependent response between CRP concentrations of 1 and 20 pg/mL. The monolayer established acceptable stability. An inconsequential response was seen when the 1:1 ratio was challenged with procalcitonin.

A rapid and sensitive electrochemical sensor has been developed for CRP, although additional optimisation is required. As CRP is just one biomarker used to diagnose sepsis, it was considered that, in the future, this system would contribute one component of a multi-biomarker sensor, used alongside other diagnostic tools, including NEWS and SOFA scores.<sup>1,3</sup>

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## **Lady's Mantle – An ancient remedy with the potential to treat modern diseases**

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Lady's Mantle (*Alchemilla Vulgaris*) is a perennial plant that has been used historically as it was thought to possess healing properties.<sup>1</sup> The aim of this project is to establish whether the plant does hold antimicrobial properties against both Gram-positive and Gram-negative bacteria. The rise in antibiotic resistance is growing into a serious public-health threat, which has caused an increasing demand for new antibiotics to be discovered.<sup>2</sup>

In the project two commercially available samples of Lady's Mantle underwent numerous extractions using water, methanol, ethyl acetate and hexane. The water extracts were tested for antimicrobial properties using zone of inhibition assays. The solvent extracts were separated out using thin layer chromatography (TLC), then any antimicrobial activity was identified using a bacterial overlay assay. The compounds exhibiting microbial inhibition were then separated and identified using mass spectrometry.

The Health Embassy sample of Lady's Mantle demonstrated activity against MSSA and MRSA in the zone of inhibition assay, but showed no activity against *E. coli*. The Mountain Fresh sample failed to demonstrate activity in the zone of inhibition assay against MRSA and only showed minimal activity against MSSA. Antibacterial activity was detected in the hexane extracts of both Lady's Mantle samples when assessed using a combined TLC/ bacterial overlay assay. The mass spectrometry result for the active compound showed no compounds other than known background compounds and plasticisers.

The plant did exhibit microbial inhibition during the zone of inhibition assay and the bacterial overlay assay, indicating Lady's Mantle does in fact possess antimicrobial properties. The activity being shown in the hexane extract suggests the compounds responsible for the activity are non-polar compounds. Further investigation needs to be done to identify the compounds responsible for the antimicrobial properties.

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## Evaluation of the Discharge Medicines Review Service

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Transitioning patients from secondary to primary healthcare settings presents many challenges.<sup>1,2</sup> Discharge from hospital is a high-risk period for medication discrepancies to occur, affecting around 14-87% patients internationally.<sup>3</sup> A medication discrepancy can be defined as any differences, intended or unintended, between two or more documented drug regimens.<sup>4</sup> The Discharge Medicines Review (DMR) service was introduced in Wales in 2011. The service involves community pharmacists identifying any discrepancies between the medication the patient was discharged from hospital with and the first prescription written by their general practitioner. A previous evaluation of the study showed that this was a well-received service overall, but barriers were affecting service delivery.<sup>3</sup> The service has not been evaluated since 2014 and the aim of this study is to evaluate the current practice of the service.

Secondary data analysis was chosen as the methodological approach. Data was obtained from NHS Wales Shared Service Partnership. The data was a combination from databases used by community pharmacies to claim payment for the service. Five researchers (L.J., I.Y., Y.C., S.C., R.R.) inputted pre-existing data from Microsoft Excel® spreadsheets into IBM® SPSS® Statistics Version 23 to be analysed descriptively.

Results showed a significant increase in the use of the service since April 2013. More contractors were DMR-accredited yet only 25% of accredited contractors were being reimbursed. Betsi Cadwaladr and Cwm Taf University Health Boards showed significant increases in their number of claims after piloting the Choose Pharmacy computer application for DMR submissions.

This study found improvement in DMR service activity, yet under-utilisation remained. The rate of discrepancies per consultation remains approximately the same as previously reported<sup>3</sup>, confirming the need for the service. Possible reasons for lack of engagement by contractors need to be explored to fully realise the services potential for improving patient safety.

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## Investigations into the Physical Stability of Neonatal Parenteral Nutrition Lipid Emulsion Syringes and Mini Bags Manufactured via a Novel Pooling Method

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Lipid emulsions are crucial components of parenteral nutrition (PN), providing a source of fatty acids to fuel the growth and development of neonates.<sup>1</sup> To help to prevent possible contamination incidents as experienced by ITH Pharma in 2014, which tragically caused three neonatal deaths<sup>2</sup>, Cardiff and Vale University Health Board (CVUHB) has decided to integrate pre-patient rapid microbiological testing into their PN manufacturing processes to improve patient safety. It was hypothesized that the ease of testing would be increased by adopting a novel pooling manufacturing method, to allow testing of multiple doses simultaneously and storing PN in mini bags to extend shelf life, providing additional time for testing. In this investigation, 10ml of reconstituted Solivito® N Infant and Vitlipid® N Infant solution and 40ml Intralipid® 20% or Smoflipid® 20% were used to make test formulations using conventional and pooling methods and the resulting 50ml syringes and 300ml mini bags (50ml unavailable) were stored at temperatures mimicking ward storage and delivery to neonates. Due to time constraints, test periods for pooling and conventional methods were 23 and 30 days respectively.

Physical stability was assessed using laser diffraction, light microscopy, visual inspection and pH monitoring. An unpaired t-test (n=3) was conducted and average percentage change from baseline at day 0 was expressed through XY scatter graphs with 95% confidence intervals.

No statistically significant differences or indications of emulsion instability such as cracking or a decline in pH below 5 were observed, therefore confirming that all PN formulations in each devices physically stable.<sup>3,4</sup>

The data illustrates that both devices manufactured by both methods were physically stable and safe for patient use for the testing periods examined by statistical analysis. Future studies should utilise 50ml mini bags to better emulate CVUHB's proposed changes to practice, in addition to possessing a longer testing duration and greater power, to reduce type II error. This investigation functions as a starting point for future physico-chemical research required to facilitate the introduction of rapid microbiological testing of PN formulations to increase patient safety.

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## The effect of simvastatin on EGFR and Src kinase family in triple negative breast cancer

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Breast cancer is the most common cancer in the UK<sup>1</sup> with the triple negative breast cancer (TNBC) occurring in 15 out of every 100 cases of breast cancer.<sup>2</sup> The lack of expression of ER, PR and HER-2 characterises TNBC and ascribes limited treatment options along with poor prognosis and outcomes to such patients. Emerging data suggests that statins may exhibit cytotoxicity in TNBC<sup>3</sup> which may involve modulation of ErbB receptors.<sup>4</sup> The aim of this study was to investigate the anti-proliferative and apoptotic effects of simvastatin in TNBC, focussing on the ErbB and Src kinase family as a potential mechanism of action.

MDA231 and MCF7 cell lines were used to model TNBC and non-TNBC breast tumour subtypes respectively. The effectiveness of simvastatin to induce cytotoxicity was analysed by the MTT assay. Western blotting was used to investigate the effect of simvastatin on TNBC cells with respect to expression of key mediators of proliferation including members of the ErbB family and Src kinase.

Simvastatin significantly inhibited the cell proliferation in MDA231 cells in a dose-dependent manner whereas MCF7 cells were not affected by this agent. Visualisation of cellular morphology confirmed this and suggested that statins induced a degree of apoptosis. Both phosphorylated (Y1086, Y1173) and total EGFR were down-regulated following simvastatin treatment whilst other ErbB members were not affected. The activity of Src (Y146) was decreased in response to statin treatment in contrast to other Src members, pLyn (Y507) and pFyn (Y530), which were not affected. Additional signalling molecules including pMAPK (T204) were also not affected by statin treatment.

We conclude that simvastatin induces tumour cell growth arrest in TNBC involving the suppression and down regulatory activities of EGFR and Src. These findings support the concept for the re-purposing of statins as a cancer therapeutic for the aggressive TNBC cancer phenotype.

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## Determination of dry biofilms presence on surfaces at healthcare settings

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A biofilm is a mostly sessile community of microbes adhered to each other and to a surface, embedded in a protective extracellular polymeric matrix.<sup>1</sup> Dry biofilms, formed in moisture-deficient environments are an increasing problem in healthcare settings due to their increased resistance to chemical disinfection and antibiotics.<sup>2</sup> Biofilms account for 65% of healthcare-associated infections (HCAI) and are affiliated with chronic infections.<sup>3</sup> The aim of this study was to determine if dry biofilms were present on surfaces at health-care settings and to identify the species of bacteria associated with them.

Samples were rinsed and swabbed under aseptic conditions and incubated in tryptone soy broth at 38°C and observed for change in turbidity. Samples were then diluted and filtered using the vacuum manifold system. Each sample was plated on 5 selective agars: MacConkey, Vogel-Johnson, multi-drug resistant-*Acinetobacter* (MDR), methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE), and incubated for 24-48h. Some samples were also analysed using scanning electron microscopy (SEM) to visually confirm the presence of a dry biofilm. DNA was extracted from the samples using Maxwell 16® instrument and quantified using the Qubit 3.0 fluorometer. Lastly, Ribosomal RNA Intergenic Spacer Analysis Polymerase Chain Reaction (RISA-PCR) was used to analyse bacterial DNA present in the samples.

Dry biofilm was demonstrated on 96% of the samples (48/50) and confirmed by SEM. Growth was attributed wholly to the biofilm due to no growth on tryptone soy agar plates after rinsing. Coagulase positive *Staphylococcus aureus* was the most identified microorganism followed by MRSA. Patient folders exhibited more growth than keyboard keys and this is hypothesised to be based on location and proximity to the patient.

Dry biofilms are present on surfaces at health-care settings and are associated with HCAs. They are affiliated with multi-drug resistant organisms and therefore a threat to patients.<sup>2</sup> Future research should focus on improved methods of detection and a more successful means of disinfection.

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## What Factors Affect the Internal Motivation of MPharm Students for the Fourth-Year Project and Third-Year Summative Assessments?

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Pharmacy is a profession which requires its students to be life-long learners.<sup>1</sup> It is important that those pursuing a career in pharmacy remain motivated to study. The aims of this research were to focus on the opinions of current MPharm 4 students at Cardiff University to gain an understanding of their motivation for their 4<sup>th</sup> year project and the 3<sup>rd</sup> year summative assessments. The main research aim was to identify the factors that affected internal motivation for summative assessments.

After gaining ethical approval, pharmacy students were invited by email for interview. Literature on Self-Determination Theory was used to develop the topic guide. Semi-structured, one to one interviews were then conducted. All interviews were audio recorded. Pilot interviews were transcribed. All transcripts were anonymised by the researchers before being thematically analysed.

The major themes identified were: OSCEs, preparedness, assessments being of relevance to future, interviewee's perception of other MPharm students' views, impact of others, enjoyment & interest, level of difficulty, and suggestions to increase motivation.

This study gives valuable insight to the opinions of MPharm 4 students about the third-year summative assessments. The suggestions provided by students, particularly of the need for feedback, may be helpful for Cardiff University to increase student's internal motivation. Furthermore, literature supporting the self-determination theory suggests that autonomy supported teaching can help increase the internal motivation of students, which can also be implemented by educators in the School of Pharmacy.

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## The design and synthesis of androgen receptor antagonists derived from the structure of bicalutamide for potential prostate cancer therapy

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In the UK, prostate cancer is the most common form of cancer in men and 1 in 8 men will get diagnosed with it in their lifetime.<sup>1</sup> The androgen receptor doesn't cause cancer but it can promote the proliferation of tumour cells so it's a target site for treatment. Currently, it is the binding site for non-steroidal anti-androgens such as bicalutamide which compete with androgens to inhibit cell growth, however resistance has developed against bicalutamide so there is a need for novel prostate cancer antagonists to overcome this.<sup>2,3</sup>

The synthesis consists of three steps. Firstly, 4-amino-3- (trifluoromethyl)benzonitrile and methacryloyl chloride react to form an amide which is then extracted and purified. The second step consists of reacting the amide with a thiophenol through Michael addition to form a sulphide derivative. Finally, the sulphide is oxidized to form a sulphone derivative. A homology model was designed to dock potential compounds in the androgen receptor and establish interactions prior to synthesising them.

Most of the sulphides and sulphones were synthesised although some consisted of impurities. One of the reactions in step 2 failed to react and due to time constraints the sulphones could not be purified. The yields for each compound varies from 16% to 92%, therefore some of the reaction conditions may need to be altered to favour a greater yield. The homology model results showed that two of the single addition compounds synthesised, docked into the pocket and could potentially have high antagonistic activity. It would be useful to test compounds in another homology model which has Arg752 instead of Arg753 before sending off for in-vitro testing.

Furthermore, the process could be replicated on an industrial scale as it is only a three-step synthesis so it is feasible if any of the compounds pass clinical trials.

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## The Structural Elucidation of New Psychoactive Substances (NPS) in Wales

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New psychoactive substances (NPS), also known by market terms such as 'legal highs', 'research chemicals', 'bath salts', and 'designer drugs', are recreational substances that mimic the psychoactive effects of illegal drugs (i.e. drugs controlled by the Misuse of Drugs Act 1971). Over the last five years, there has been an unprecedented increase in the number, type, and availability of NPS, presenting a significant challenge to healthcare, as well as law enforcement and policy.<sup>1</sup>

The WEDINOS project, funded by the Welsh Government, is a harm reduction service that allows established or prospective drug users (or their representatives) to submit drug samples anonymously for chemical structure analysis.<sup>2</sup> In this report, five NPS samples submitted to WEDINOS were structurally elucidated, quantified (if mixture of multiple constituents) and identified using time-of-flight mass spectrometry (TOF-MS) and nuclear magnetic resonance (NMR) techniques. The information obtained was used to investigate the purported 'lack of' Quality Assurance/Quality Control (QA/QC) associated with NPS, and the subsequent effect this has on the health/safety of consumers.

The results of spectroscopic analysis revealed that, of the five samples tested, all five had notable defects relating to a 'lack of' QA/QC processes. This included, but was not limited to: presence of impurities (3), unknown structure(s) (1), illegal contents (1), dosage non-uniformity (1) and not as advertised (2). Four of the five samples also contained one, or more, active ingredients in which limited (if any) pharmacological and toxicological data exists.

It was concluded that the investigation illustrates the variability and uncertainty surrounding the contents of each NPS product. It also demonstrates the relative vulnerability of consumers that purchase NPS; they instill a level of trust into their respective sources without fully realising the legal (prosecution) and/or health related (hospitalisation/death) implications.

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## Design and Synthesis of Potential CtIP-1 Inhibitors by Structural-Based Lead Optimisation

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Cancer is a disease caused by an uncontrolled division of abnormal cells which can occur in any part of the body. This project is intended to research novel approaches of inhibiting the transcriptional regulator C-terminal binding protein 1-interacting protein (CtIP) protein. CtIP is an important protein in the process of homologous recombination, a pathway by which the cell can repair double stranded breaks<sup>1</sup>. Inhibiting the CtIP protein is a novel approach at sensitising cancer cells to DNA damaging agents (like platinum salts<sup>2</sup>), which is needed due to the resistance mechanisms displayed by cancer cells to these agents. The aims of this project are to synthesise and investigate a number of potential inhibitors

Screening of CtIP protein with 300,000 commercially available compounds was undertaken and the further narrowing of the results, including 3 further assay studies, revealed 2 'hit' inhibitor compounds for the CtIP target. Consequently, a range of potential inhibitors were synthesised, using structural-based lead optimisation of one of these 'hit' compounds. Further, molecular docking studies of potential inhibitors with the CtIP target was undertaken to investigate the receptor binding.

10 potentially optimised CtIP inhibitors were synthesised as well as the 'hit compound' with varying yields. Molecular docking studies revealed there are 3 potential major binding positions and the key interactions for these positions have been studied.

Although there is one compound currently undergoing clinical trials that targets CtIP<sup>3</sup>, there are none that use this novel approach. Successfully synthesised compounds in this project are to be sent to the laboratory of Dr Staples (School of Medical Sciences, Bangor University) for testing in biological assays so that their activity can be further understood.

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## How Does the Temperature of a Drink Used When Swallowing Capsules Affect the Shell Dissolution Rate of Both HPMC and Gelatin Capsules?

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Oral drug delivery offers several advantages and comes in many forms, one of the most common forms being capsules, which are commonly comprised of gelatin or, in recent years, can also be HPMC (hydroxypropyl methylcellulose). One factor to consider when administering a drug orally is the temperature of a drink used when taking an oral solid dosage form and how this affects its ability to dissolve to release the active pharmaceutical ingredient. The objectives of this study were to identify how hot and cold drinks which are a different temperature to the body may affect capsule dissolution and ultimately how this may affect the delivery of a drug.

Initially, the average temperature of hot and cold drinks was identified in the literature. Liquids at these temperatures were added to fixed volumes of dissolution medium, set at 37°C, that represent stomach fluid volume in the fasted and non-fasted state. The changes in fluid temperature and time taken for the fluid to return to 37°C was measured. HPMC and gelatin capsules were then placed in dissolution medium at 24°C, 37°C and at 48°C, as guided by the above experiment. Finally, the researcher measured how different temperatures affected the dissolution rate of encapsulated paracetamol from both HPMC and gelatin capsules using UV-Vis spectrophotometry.

Results showed that whilst the disintegration rate of HPMC capsules were unaffected by an increase in temperature, the disintegration rate of gelatin capsules increased with temperature. Results also showed that the dissolution rate of encapsulated paracetamol increased with temperature for both HPMC and gelatin capsules, demonstrating how the drink taken with a capsule may influence drug release characteristics.

## Using Technology to Improve the Health of Community Pharmacy - What is the best method of training and support?

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Technology is changing the role of the community pharmacist by providing a greater arsenal of tools.<sup>1</sup> As such robust training regimes should be available to enable pharmacies to maximise the advantages technology can provide. In instances of inadequate training with IT systems it can result in more errors which poses a risk to the patient.<sup>2</sup> The aim of this study was to explore current methods of Information Technology (IT) training and support available to community pharmacists and investigate how these methods can be refined in the future.

A qualitative methodology was used in this study utilising semi-structured focus groups held with numerous pharmacists purposively selected due to their backgrounds in community pharmacy varying and as such will

bring about various discussion points and views.<sup>3</sup> Focus groups were transcribed *ad verbatim* and thematically analysed using an inductive methodology.<sup>4</sup>

In total eleven participants were interviewed over two focus group sessions with a total of six themes being identified after thematic analysis, each was then broken down into sub-themes. The six main themes identified were namely 1) Training Methods, 2) In-Store Responsibility, 3) Locum / Multi-Branch Pharmacists, 4) Customer Support, 5) Customer Success, and 6) Ongoing Feedback.

Results show the current standard of training and support offered by Patient Medication Record (PMR) system providers is perceived to be inadequate. PMR systems consist of many distinctive features integrated into one system enabling many services. As such the training provided will dictate how effectively the software system is onboarded and determine whether the most is made out of the software's features. Support mechanisms are commonly only used when problems arise, but expectations from some of our participants showed that as customers they expect more going forward, in order for them to provide optimised patient care through technology.

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## Spontaneous Reporting of Adverse Drug Reactions: Barriers and Facilitators for Dentists

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Yellow cards are part of a vital pharmacovigilance mechanism developed by the Medicines and Healthcare products Regulatory Agency (MHRA) in which Adverse Drug Reactions (ADRs) can be spontaneously reported via the Yellow Card Scheme. Only one Yellow Card was submitted to Yellow Card Centre Wales in 2016/17 by a dentist which equates for just 0.04%.<sup>1</sup> In previous research, it has been found that there was a lack of knowledge regarding ADR reporting<sup>2</sup> and additional training needs were identified.<sup>3</sup> This project aims to explore the views and experiences of dentists on spontaneous ADR reporting including the barriers they face, potential facilitators to increase reporting and any previous training received.

Qualitative research methods were adopted due to the explorative nature of the project. Snowball sampling was used to recruit participants. To be included in the study, the dentists had to be working in a community based practice. A semi-structured interview schedule was used in the interviews which contained open questions and prompts. Interviews were conducted face-to-face or by telephone, audio recorded then transcribed *ad verbatim*. Thematic analysis was then used to identify major themes in the data. Ethics approval was obtained for the project.

Six interviews were carried out; five face-to-face and one via telephone. Some of major barriers identified included time pressures, payment of dentists, undergraduate teaching, knowledge and experience. Examples of possible facilitators identified were more training, continuing professional development and making Yellow Cards easier to complete.

The results showed that dentists experience multiple barriers to reporting, however recommendations to facilitate these changes were also made. Further research into this area would allow better understanding to the issues across Wales. Suggestions for improvements have been made and hopefully the amount of spontaneous ADR reporting will increase in the coming years.

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## Stability of Medication in Multi-Compartment Compliance Aids Administered by University Hospital, Wales

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Multi-compartment compliance aids (MCAs) are devices used to deliver solid dosage form medication to patients via a series of plastic compartments, aiming to match a patient's dosage regimen and ease administration.<sup>1</sup> Despite their popularity, there is a paucity of research into the stability of medications placed in an MCA. Their use involves removing a product from its primary packaging and therefore invalidating the manufacturer stability guarantee. Medications can be degraded by light, moisture, air and heat.<sup>2</sup> Degradation of a medicine could lead to changes in active ingredient concentration, degradant accumulation, drug release profile or visual appearance. Consequences for a patient could include under-dosing, toxicity or non-compliance.<sup>3</sup> The aim was to use Specialist Pharmacy Service (SPS) database to analyse a sample of UHW MCA prescriptions and determine the number of potentially unstable items that were dispensed. Another aim was to make risk reduction recommendations based on results and surrounding evidence.

UHW prescription data spanning 6 months was anonymised and assigned stability codes through SPS, an online resource allowing product stability information to be shared between healthcare professionals. The frequency of each allocated code was analysed, as well as the formulation and environmental sensitivities.

The results showed that 17% of items dispensed into the MCAs were deemed unsuitable by SPS, affecting 66% of MCAs recorded. Dispersible aspirin tablets, levothyroxine tablets and amlodipine tablets made up 49% of all unstable items identified and were the focus of the resulting recommendations.

Based on results and surrounding literature reviews, recommendations were made to reduce the number of unstable items and the risk of degradation of items in MCAs. These included educating both patients and professionals and maintaining optimum storage conditions throughout MCA assembly and administration. This research also highlighted the need for further stability testing to produce National guidelines for medication storage and use in MCAs.

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## To investigate the factors that influence final year MPharm students' motivation in relation to third year summative assessments and the early stages of the final year project

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Several theories exist that seek to explain motivation. According to self-determination theory (SDT), there are several types of motivation; amotivation, intrinsic and extrinsic. These all vary in the degree of autonomy. Intrinsic motivation is the highest form of motivation and has an internal perceived locus of causality. Motivation is maintained or enhanced by satisfying the psychological needs for autonomy, competence and relatedness. Intrinsic and internal motivation results in deeper learning, better academic performance and overall wellbeing.<sup>1,2</sup> SDT has been applied in many different fields, including education, however little research has been done in pharmacy context. The aim of the study was to explore the factors that influence final year MPharm students' motivation in relation to third year summative assessments and the early stages of the final year project.

Semi structured, one-to-one interviews were conducted to explore the views of fourth year MPharm students. A topic guide, along with an interview schedule, consisting of mainly open-ended questions, were constructed to guide the interviews. Non-probability, purposive sampling with an element of convenience was used to recruit students. Interviews were audio recorded and transcribed verbatim. Thematic analysis<sup>3</sup> was used to

analyse data. Themes were derived inductively, and deductively using SDT. Approval from Ethics was obtained.

A total of 18 interviews were conducted. Students identified factors that influenced their motivation in relation to their final year project and third year summative assessments. Six major themes, and several subthemes, were identified. Major themes were final year project, preparedness, influence of lecturers, choice, group work and relevance.

This study has helped provide a valuable insight into fourth year MPharm students' views at Cardiff University. In addition, based on these findings, suggestions have been made to the School to help increase internal motivation. Although, results are not generalisable, it has provided the basis of further research.

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## Exploring RET expression in breast cancer

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Breast cancer is the UK's most common female cancer with several successful anti-hormonal (AH) treatments, for ER+ patients; however, resistance and relapse remains a major problem.<sup>1</sup> Mechanisms of resistance have been investigated, preclinical models revealing an importance for erbB pathways<sup>2</sup>, but their targeting has had limited clinical success. RET, a further tyrosine kinase, is a newer target, with promising research showing increased signalling in a tamoxifen-resistant cell line.<sup>3</sup> It is now the target of a clinical trial (FURVA) exploring a kinase inhibitor, vandetanib. The key objectives here were to optimise a RET immunohistochemical (IHC) assay and use it to determine any signalling or clinicopathological associations for RET in breast cancer patients, and to study further pre-clinical models to explore relationships between RET and AH resistance.

A RET IHC assay was optimized successfully using microwave antigen retrieval (pH9 EDTA buffer) that could adequately-stain stored breast cancer sections. It was applied to a historical patient series (n=93) and to a panel of AH-resistant cell pellets and xenografts. RET staining was evaluated by H-Scoring. Statistical analysis examined relationships between RET staining and biological and clinicopathological data available for the patients.

RET correlated with its co-receptor GFRA1 (p=0.003), and while inverse with erbB signalling, positively correlated with STYK1 (p<=0.001) and downstream signaling including Shc (particularly in ER- disease, p=0.012) implicated in AH resistant phenotypes. Higher RET levels associated with increased tumour size (p=0.03) and recurrence (p=0.005). All cell lines and xenografts stained for RET, particularly an oestrogen-deprived resistant model.

Although the samples were >10 years old and storage effects antigenicity<sup>4</sup>, data supportive of RET signalling and its relationship to AH resistance were obtained from the clinical breast cancers and the xenograft and pellets. The direct associations with adverse clinicopathological features affirmed that RET overexpression is undesirable, advocating trials like FURVA.

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## Factors associated with falls in a convenience sample of patients and the public presenting at community pharmacies in Wales

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The causes of falls are multi-factorial<sup>1-3</sup> and include certain medication<sup>2</sup> and increasing age.<sup>3</sup> The study aimed to identify if there is any statistical association between pre-determined risk factors and a fall occurring within the past year based on a sample of Welsh community pharmacy patients.

Data were collected via a questionnaire by community pharmacies as part of a national falls campaign conducted in Wales in 2017. The questionnaire focussed on the presence or absence of some associated risk factors.<sup>1-3</sup> Ethical approval was granted by Cardiff School of Pharmacy and Pharmaceutical Sciences Ethics Committee. This project involved coding, data entry and analysis of the completed questionnaires. Following validation of data, the chi-square test was used to compare risk factors with whether or not the individual had experienced a fall in the previous year.

A total of 4630 questionnaires were received with 36% of respondents reporting a fall in the past 12 months. Individuals taking a number of specific medicines/classes of were significantly more likely to have reported a fall in the past year. There was a statistically significant higher proportion of falls reported by respondents over 70 years old compared with younger respondents. Individuals taking four or more medicines (polypharmacy) were significantly more likely to have reported a fall in the past year than those taking three medicines or fewer.

This study found that certain medications, age and polypharmacy were associated with an increased risk of a fall in the previous 12 months in the community setting in Wales. These findings align with those of others. Suggestions for changes to the questionnaire have been made. Further research is warranted.

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## Synthesis of haemanthamine derivatives as potential leads for drug discovery

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Galantamine, a drug used to treat mild to moderate Alzheimer's Disease<sup>1</sup>, is commercially harvested from daffodils.<sup>2</sup> The extraction process results in the generation of many by-products some of which have been shown to have biological activity. Haemanthamine, one of the by-products resulting from the extraction of galantamine has been shown to have anticancer activity. Specifically, haemanthamine shows effects against apoptosis resistant cancers which are currently impervious to treatment.<sup>3</sup> Haemanthamine displays sub-optimal lipophilicity (LogP) for oral dosing<sup>4</sup>, a surrogate for polarity which can determine the amount of haemanthamine reaching the systemic circulation and its rate of excretion from the body. The aim of this project was to make novel analogues of haemanthamine that improve its properties for oral administration. These structural analogues could then be used as potential leads for drug discovery.

Three chemical reactions were designed to improve the lipophilicity of haemanthamine from a LogP value of 0.91 to within the range of 1 to 3. These were, an esterification to make a butyl ester of haemanthamine, an ether synthesis to make a butyl ether of haemanthamine and lastly a methylenedioxy cleavage reaction. The reactions were run and their degree of success judged via thin layer chromatography (TLC), mass spectrometry and proton nuclear magnetic resonance (1H NMR).

Both the esterification and ether synthesis reaction were successful as confirmed by 1H NMR. The methylenedioxy cleavage reaction proved slightly harder and was inconsistent.

The esterification and ether synthesis reactions both showed very positive results, and were highly reproducible. The methylenedioxy cleavage reaction requires further work to establish reproducibility.

The ester and ether products of haemanthamine are likely to serve as potential useful leads for drug discovery.

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## Pharmacists' knowledge and understanding of the use of Image and Performance Enhancing Drugs (IPEDs) in sport

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Doping is the use of any prohibited substances and methods determined by the World Anti-Doping Agency to enhance performance in amateur and professional sport.<sup>1</sup> There has been little research to date regarding pharmacists' knowledge on the use of IPEDs, pharmacy medicines containing banned substances and the role of pharmacists in addressing this issue.<sup>2,3,4</sup> The aim of this research was therefore to identify practicing pharmacists' knowledge of IPEDs and their perceived need for additional training on this subject.

Ethical Approval was granted by the School's Research Ethics Committee. Research was conducted over two phases: (1) optimisation of an existing questionnaire and (2) questionnaire distribution to pharmacists. In phase 1, two focus groups were conducted with teacher practitioners at Cardiff School of Pharmacy to determine the feasibility of the questionnaire, appropriateness of questions and ease of completion. The feedback aided finalisation of an anonymous postal questionnaire comprising a range of Likert-scale, ranking and open and closed questions. The questionnaire was sent to 494 pharmacies across all Health Boards in Wales. Participants were given two weeks to respond. Data was analysed using SPSS.

119 completed responses were received (response rate = 24.3%) The majority of pharmacists (88%) were unable to correctly identify banned substances within common over-the-counter medicines. Similarly, 86% of pharmacists identified as feeling 'unsure' or 'not confident' on advising the public and athletes on IPEDs. Despite this, 58% of respondents felt pharmacists should be one of the first healthcare professionals to offer advice on this issue, demonstrating a need for further training, for which 80% stated they would be interested to receive.

The results obtained through our questionnaire corroborated data in France and Cameroon, with pharmacists' feeling poorly prepared and lacking confidence. However our results regarding advising on IPEDs did not align with those obtained elsewhere, therefore further research is required.

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## Optimisation and biocompatibility of alginate gel for 3D bioprinting

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3D bioprinting is the accurate placement, via spatial control, of encapsulated cells to produce 3D structures.<sup>1</sup> Commercial 3D bioprinters are currently expensive and often sub-optimal and consequently access to this technology is limited. The bio-inks utilised to print cells are an important feature of the system. This project aims to develop an affordable bio-ink that is compatible with skin cells and thus could be used to 3D print a human skin substitute.

Alginate was selected as the lead candidate bio-ink. It is affordable and approved by both the FDA and MHRA for use in medical products.<sup>2</sup> Gelling methods were optimised, with the intention of testing the biocompatibility of alginate with two types of skin cell, fibroblasts and keratinocytes. Alginate was gelled in a 12-well plate at a range of thicknesses, before cells were seeded onto the surface of the gel. Cells were inspected regularly, and growth was monitored over 7 days. The functionality of alginate as a bio-ink in a bespoke 3D printer was then tested. The gel was used to print fibroblasts in different spatial orientations, as programmed into the printer.

Alginate can be gelled in various ways. However, many substances used to gel alginate can influence the growth of cells. Method optimisation in gelling alginate allowed for some removal of these substances e.g. mineral oil, thus increasing the chances of survival of cells. Alginate was demonstrated to be biocompatible, supporting the growth of both fibroblasts and keratinocytes. This allowed the bio-printing of multi-layered shapes, showing proof of concept.

Various grades of alginate are available with different G/M ratios and therefore the effect on biocompatibility should be explored. Further optimisation of the alginate is required and future research should, investigate the ability of alginate to support less robust cell lines such as melanocytes.

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## Investigating expression of plasmid DNA and messenger RNA in model human skin cells.

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The use of plasmid DNA (pDNA) and messenger RNA (mRNA) as vaccines has been of recent interest due to eliciting a strong immune response by activation of cytotoxic T cells, T helper cells and antibodies.<sup>1</sup> It is a step forward in developing modern vaccine technology to overcome limitations<sup>2</sup> with traditional vaccine therapy. Investigating expression of genes in HaCaT cells provides knowledge on how gene vaccines, when delivered into the epidermal layer of the skin, will behave.

This study investigates the effectiveness of the transfer and the expression of genes coding for green fluorescent protein (GFP) into HaCaT cells via mRNA/pDNA transfection. HaCaT cells were cultured in a low-calcium medium, representative of conditions in the lower epidermis of human skin. Following this, transfection of pDNA and mRNA, coding for GFP, with and without delivery systems were conducted. Expression of GFP caused by pDNA and mRNA was compared and analysed using fluorescence microscopy and flow cytometer.

Comparison of the two gene vaccines using analytical techniques indicated that expression of GFP was more prevalent when pDNA was combined with Lipofectamine 3000. Adjusting concentrations to investigate cell death resulted in a decrease of MessengerMAX to increase GFP expression, although no significance difference was seen with other alterations. Comparison against controls measured the desired expression, excluding undesired expression, to produce results that can contribute to the field.

Results produced provide initial data for further research into the development of gene vaccines using primary cells. It has shown that in low-calcium HaCaT cells pDNA and Lipofectamine 3000 is the most successful at inducing GFP expression when in comparison with mRNA and MessengerMAX.

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## Design and synthesis of novel aspartyl tRNA synthetase (AspRS) dual inhibitors as novel antibacterial agents

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Antibiotic drug resistance is becoming an increasing global problem, resulting in the demand for urgent discovery of alternative antibacterials. One such example is aminoacyl tRNA synthetase (aaRS) inhibitors which target the core process of protein synthesis needed for cell survival.<sup>1</sup> This report focuses on aspartyl tRNA synthetase (AspRS) enzymes, with the aim to design and synthesise novel dual inhibitors as an alternative to current treatments.

In order to design the compounds the enzyme active site was analysed using molecular modelling software (MOE<sup>2</sup>). The software provided an insight into binding interactions between the natural ligand and enzyme which enabled the composition of the compounds to be determined with the intention to fill two pockets - the ATP site and the aspartic acid site. The synthesis of the compounds was a four-step reaction: nucleophilic substitution to form a sulphonamide bond, nucleophilic substitution with chloroacetonitrile, reduction of nitrile to amine using LiAlH<sub>4</sub> and amide bond formation using an acyl chloride. The products were confirmed using mass spectrometry, <sup>1</sup>H NMR and <sup>13</sup>C NMR.

Two compounds were synthesised with the exclusion of the linker group. Step 2 was partially optimised but needs further research to prove the reliability of the method. Similarly, an alternative method for step 4 was suggested but also needs confirmation of its suitability.

Although none of the final compounds were produced during the project, there has been useful development of the methods and an opportunity for further optimisation of the reaction steps.

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## Investigating the effects of Alzheimer's disease, gender and age on the expression of mitochondrial proteins in the human brain

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Alzheimer's disease (AD) is the most common form of dementia.<sup>1</sup> The pathophysiology of the condition involves the accumulation of amyloid beta plaques and neurofibrillary tangles.<sup>2</sup> Mitochondrial proteins of the electron transport chain (ETC) also play a crucial role in the progression of AD.<sup>3</sup> The aim of this study was to make comparisons of the expression of these mitochondrial proteins between disease states, genders and age groups.

The semi-quantitative, analytical method known as Western blotting was used for the separation of mitochondrial proteins from human brain samples. All proteins were imaged using chemiluminescence. Relative densities of bands from the images were determined using Image J. Bands were normalised to GAPDH or total Dynamin-related protein 1. Data were statistically analysed using Student's t-test and ANOVA.

The expression of complex II and IV demonstrated a significant decrease in AD compared to non-AD male brain samples. An overall trend for a decrease was seen in the expression of all complexes in female old compared to male old, and female AD compared to male AD brain samples. The expression of complex V demonstrated a significant decrease with age in female brain samples. An alteration in the expression of

dynamain-related protein 1 was observed between age groups and disease states in both male and female brain samples.

In AD and age groups, more consistent trends were observed in ETC complex expression in women compared to men. This is likely to be related to the decline in oestrogen levels in postmenopausal women leading to enhanced amyloid beta production and decreased mitochondrial function. The findings suggest mitochondrial proteins likely play a key role in AD pathophysiology. Understanding the link between these proteins and oestrogen may help to develop treatment strategies by reversing amyloid beta-induced mitochondrial dysfunction.

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## **Transfection of High Calcium Culture HaCaTs with Gene Vaccines (pDNA and mRNA) Encoding Green Fluorescent Protein (GFP)**

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Gene vaccines plasmid DNA (pDNA) and messenger RNA (mRNA) present a promising vaccine technology. (1) However, in practice they fail to stimulate potent immune responses.<sup>1</sup> Keratinocytes in the epidermis are in close proximity to large numbers of immune cells, their role in immune cell recruitment suggests their transfection with gene vaccines may secure highly potent immune responses.<sup>2</sup> However, to date this has not been widely investigated. This project investigated transfection of a differentiating keratinocyte model with pDNA and mRNA. A concurrent project investigated transfection of a proliferative keratinocyte model. It was also investigated whether enhanced transfection efficiency of gene vaccines would be achieved in presence of lipofectamine, a gene delivery system.

A keratinocyte model, immortalised human keratinocytes (HaCaTs) were used. Differentiated HaCaTs were achieved through culturing cells in high calcium (2.8mM) medium. Genes encoding green fluorescent protein (GFP) were used thus successful transfection of HaCaTs was indicated by expression of GFP. GFP was visualised with epifluorescent microscopy and quantified with flow cytometry.

pDNA only in presence of a delivery system successfully transfected differentiated HaCaTs. However, in contrast to the results of a concurrent project, transfection success was minimal.<sup>3</sup> Results following transfection of HaCaTs with mRNA were inconclusive. Due to time constraints optimal transfection conditions could not be investigated. Previous studies state that unlike pDNA, nuclear entry is not required for mRNA expression, thus greater transfection efficiency would be predicted in non-replicating cells such as differentiated HaCaTs. (4) Furthermore, unlike pDNA, mRNA does not present a risk of integrating with the cells genes. (4)

mRNA based vaccines theoretically present a better vaccine technology as opposed to pDNA.<sup>4</sup> However, whether high transfection efficiency in epidermal skin cells can be achieved and whether administration of gene vaccines into the epidermis would secure potent immune responses would require further investigations.

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## Assessment of Inhaler Technique and Asthma Control in a Community Pharmacy Setting

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Asthma control remains poor in Europe, with studies showing 45% of patients have uncontrolled asthma.<sup>1</sup> Many contributing factors can be considered, including: one third of asthmatic patients do not use their inhalers correctly, less than 50% adhere to their preventative inhalers and only 7% of healthcare professionals were found able to demonstrate all the steps required to use a MDI correctly and effectively.<sup>2-4</sup> The aim of this study is to assess asthma control and inhaler technique.

Sixty-one patients were recruited using convenience sampling across four community pharmacies in South Wales. Following consent, a semi-structured interview approach was used to collect biometric data including: age, smoking status, past inhaler technique training and history of hospitalisation/oral steroid use due to asthma exacerbations. Asthma control was measured using an Asthma Control Test (ACT). Inhaler technique was assessed using an Aerosol Inhalation Monitor (AIM device, Vitalograph).

The main findings were that 55% of participants had uncontrolled asthma in the past four weeks, based on the ACT. Moreover, 33% had been hospitalised or prescribed oral steroids due to asthma exacerbations, an outcome of severe asthma. Overall, 53% of participants failed their inhaler technique test when using the AIM device. The largest number of participants, 61%, demonstrated the wrong technique when use a metre dose inhaler (MDI). The majority of participants received inhaler technique training from a doctor or nurse. Only 5% received training from a pharmacist and 13% received no inhaler technique training from any health care professional (HCP).

Effective self-management should be encouraged among asthmatic patients. HCP training on inhaler technique should be reviewed so that they can effectively and confidently train patients. Shorter review intervals could help facilitate regular assessment and reinforcement of inhaler technique. Pharmacist intervention in asthma care can prove to be beneficial and should be studied further.

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## Why are manganese and selenium plasma levels not in the optimal range in Home Parenteral Nutrition (HPN) patients?

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HPN provides nutrients to patients at home, to meet their nutritional needs when the oral route is inaccessible.<sup>1</sup>

<sup>2</sup> Additrac® is a multi-trace element product that has been used for many years to provide micronutrients to patients. A previous study has shown that long-term HPN patients have exhibited some abnormal trace element (TE) blood results.<sup>3</sup> The aim of this study is to review prescription data and blood test (BT) results for PN patients to determine whether their prescriptions are optimal or if there is scope for improvement.

The TEs targeted in this study were manganese (Mn), selenium (Se), zinc (Zn) and copper (Cu). All patients were anonymised, and their PN bags were added either with "Additrac® only", "extra Se only" or "both Additrac® and extra Se", thus divided into three groups. BT results were taken minimum 3 months after initiating the PN regimen, to allow TE levels to be consistent in the body. The results were recorded and categorised as "deficient", "in range" or "in excess". Data analysis was then carried out.

Of the 4 TE, most patients did not have "in range" BT results for Mn and Se, hence for further investigations were undertaken. Statistics test was conducted, with the null hypothesis 'There is no relationship between Mn and Se'. The highest correlation coefficient of -0.294 in "Additrac® only" indicated that Mn and Se have a

strong relationship. Although the test did not indicate whether Mn or Se was the contributing factor to abnormal plasma levels, they were closely related to each other when only Additrac® was added into the PN bag.

Although the relationship was strong, due to the small sample size, the null hypothesis was not rejected. Therefore, future work must be performed to include more BT of “Additrac® only”, such as increasing the sample size in the study.

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## How temperature and formulation factors affect precipitation in neonatal aqueous parenteral nutrition admixtures containing copper and zinc

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Parenteral nutrition (PN) contains water, amino acids, glucose, lipid emulsions, electrolytes and micronutrients. This provides nutritional support to patients with malnourishment risk, due to medical conditions or diet inadequacy.<sup>1</sup> Precipitations in PN can cause patient harm by particulates blocking capillaries and/or result in inadequate quantities reaching patients. This project's aim was to analyse whether a PN admixture's stability was affected by copper and/or zinc additives when combined with amino acids Vaminolact® (containing L-cysteine) or Aminoven 25®, potentially contributing to precipitation development in test admixtures.

Using a laminar flow unit, admixtures containing various volumes of glucose and amino acid solutions together with copper and zinc dilutions were prepared. Visual inspection, turbidity and pH levels were analysed for admixtures stability. Testing occurred on preparation day (0 hour) at room temperature and at 24, 48 and 72 hours in a stability chamber at 30°C and 35°C. This replicated neonatal PN administration in hospitals.

Under fibre optic lighting, admixtures containing solely zinc additives, remained clear and colourless whilst those containing only copper additives produced a blue tinge (BT). Both these admixtures maintained stability according to turbidity and pH level data. A yellow haze (YH) occurred only when copper and zinc sulfate additives were in combination. The tyndall effect, indicated that particulates were present in BH and YH admixtures.<sup>2</sup> Stability in YH admixtures could not be ensured as turbidity values resulted in significant differences of >0.5.

The YH micro-precipitates could be products formed from reactions involving copper and either cysteine or hydrogen sulfide (by-product of cysteine degradation).<sup>3,4</sup> Glucose had a protective effect, slowing YH's development. Temperature increased solubility within the solution, potentially allowing particulates to re-disperse. Future chemical stability analysis would be advised alongside using a particle counter and Scanning Electron Microscopy with X-ray Energy Spectroscopy (SEM-XES) to assess the size and identity of these micro-precipitates.<sup>4</sup>

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## Discovery of potential inhibitors for Chikungunya virus using computational approaches

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There have been several Chikungunya virus outbreaks over the last few years with there being more than 1.7 million suspected cases of it in affected areas since 2013. Contraction of the virus can lead to acute flulike symptoms and in some cases to chronic debilitating joint pain. Infants and the elderly are more likely to experience severe symptoms which in rare cases can lead to death. Currently there is no drug treatment or vaccine for the virus on the market but the macro domain contained within the non-structural protein, nsP3, is a potential target for antivirals.<sup>1</sup> The aim of this project is to identify potential inhibitors for this macro domain using computational molecular design.

The co-crystallised structure of the macro domain with ADP-ribose was downloaded from Protein Drug Bank (PDB ID: 3GPO) to Molecular Operating Environment (MOE). Three pharmacophore queries were generated to screen the SPECS library and the PCL fragment library. The resulting molecules were docked into the Chikungunya macro domain using Glide SP. They were then rescored using Glide XP, FlexX and Protein Ligand ANT system (PLANTS). A consensus score was taken including only the top 25% ranked molecules across all 3 scoring functions. Visual inspection was then performed on the resulting 498 poses.

13 unique molecules were chosen in the final selection. To ensure that these molecules didn't bind well with human macro domains they were docked into the crystal structures of 4 different human macro domains and rescored with Glide XP, FlexX and PLANTS to calculate a consensus score. 2 of the molecules showed some indication that they could bind well with the human macro domains however after investigating their protein-ligand interactions they were deemed suitable to be taken to the next stage.

The obtained molecules might present new Chikungunya virus inhibitors with none or only little effect on human macro domains. Further cell-based evaluation is needed to assess the anti-Chikungunya virus activity and to confirm the computational results.

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## Exploring ways in which internal motivation of MPharm students can be increased in third year summative assessments from the perspective of current fourth years.

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Three main psychological needs of autonomy, competence and relatedness must be met in order to develop intrinsic motivation which can then enhance performance.<sup>1</sup> According to the self determination theory (SDT), intrinsic motivation can optimise enjoyment and interest for a student which as a result can benefit them academically by encouraging deeper learning rather than surface learning.<sup>2,3</sup> An extrinsically motivated student is motivated by factors like rewards or punishments.<sup>2</sup> The main aim of this study is explore factors that increase internal motivation in assessments and suggest improvements for the school of pharmacy to consider in trying to increase internal motivation.

Non probability (convenience and purposive) sampling was used to recruit fourth year Cardiff MPharm students. They were asked to take part in a semi structured interview which was audio recorded and ethically approved. The interview explored what motivation was for individuals and their fourth year projects. They were required to rank third year assessments into three piles of most, middle and least, according to enjoyment/interest and also separately by relevance to the MPharm degree and/or after graduation and provide reasons. All anonymised transcripts were accuracy checked and manually coded using thematic analysis.

Five major themes were identified from transcripts of 18 interviews; ability of having a choice in topic, placements, relevance to future, influence of others and preparation and guidance. Students suggested changes that could be made to increase or maintain their internal motivation for assessments.

Changes that were suggested fit within the SDT and have the potential to fulfil the needs of autonomy, competence and relatedness. However, not all changes would be feasible to do and would have to be further investigated in order to be carried forward. More research would need to be done on feedback and topic choice in order for the school to adopt these suggestions.

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## **Influence of material and surface properties of orthopaedic biomaterials and its coatings on bacterial adhesion.**

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Bone and joint disorders such as Osteoarthritis affect individuals all around the world and at least 8 million people in the UK alone.<sup>1</sup> The prevalence is continuing to rise along with obesity and the ageing population. Therefore, increasing the number of total joint replacement (TJR) procedures being performed, giving rise to an increase in the number of infection cases.<sup>2</sup> This study aims to explore material and surface characteristics of biomaterials and coatings used to make joint implants and how these properties influence bacterial adhesion.

Surface adhesion parameters for biomaterials, bacterial strains and coatings relevant to orthopaedic infections were found from literature. They were integrated into a computer stimulation model called a multi-asperity adhesion model and stimulations were executed using a programme called 'Force 2.' This model was derived from the Johnson, Kendall and Roberts (JKR) model, a single-asperity model assuming that one surface is smooth (bacteria) and another surface is rough (material).<sup>3</sup> The output data presented the adhesive interactions between bacteria and materials.

A statistically significant difference ( $p < 0.05$ ) was found for adhesive force averages across all materials. Ultra-high-molecular-weight polyethylene was found to be the most adhesive with an overall average of ( $52.8 \pm 5.7 \text{ nN}$ ) and aluminium oxide was found to be the least adhesive ( $35.9 \pm 6.5 \text{ nN}$ ). Findings on coatings demonstrated that chitosan and silver lowered adhesive force even further.

Material and surface characteristics such as Poisson's ratio ( $\nu$ ), elastic modulus ( $E$ ) and surface energy ( $\Delta\gamma$ ) were found to influence bacterial adhesion. It was recognised that highly elastic materials were less adhesive to bacteria and bacteria with low surface energies were more adhesive towards hydrophobic surfaces. All of which justified the importance of surface properties and the effect they have on bacterial adhesion. Therefore, material and surface properties of joint implants can influence the prediction and prevention of orthopaedic infections.

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## **Design and synthesis of novel human Norovirus RNA-dependent RNA polymerase inhibitors**

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Norovirus is a small, non-enveloped, positive-stranded RNA virus belonging to the Caliciviridae family. This virus is a worldwide threat and is responsible for almost 50% of gastroenteritis cases. It is associated with increased chance of hospitalisation and mortality.<sup>1,2</sup> Currently, there is no effective treatment or pharmacological prophylaxis for Norovirus, generating an urgent need for an antiviral therapy.<sup>2</sup> RdRp (RNA-dependent RNA polymerase) is a protein present within Norovirus and has an essential role in synthesis and

amplification of additional subgenomic RNA, as well as in genome replication.<sup>3</sup> This protein is suggested to be a promising antiviral target due to its crucial role in the survival of Norovirus. Furthermore, unlike other proposed antiviral targets, research into this protein has identified its crystal structure.<sup>2</sup> This study involves molecular docking and synthesis of potential antivirals to target Norovirus RdRp, with an aim to inhibit this protein.

A library of compounds was constructed on a molecular modelling software, exploring the structural activity relationship and making modifications to lead compounds 1 and 2, to improve their effectiveness and activity. These compounds were previously discovered as inhibitors of Norovirus RdRp through virtual screening. Analogues were designed and then docked within the crystal structure of RdRp, and each configuration was ranked according to its docking score. Taking this score into consideration, all configurations were visually inspected assessing its key interactions within the protein binding site, as well as comparing the fit to the known inhibitor of RdRp, PPNDS (pyridoxal-5'-phosphate-6-(2'-naphthylazo-6'-nitro-4',8'-disulfonate) tetrasodium salt).

After computer analysis, the most promising compounds were chemically synthesised. 14 compounds were successfully prepared and were all over 95% pure.

All of these compounds are currently being biologically evaluated against Norovirus RdRp activity. The results from this study's computer modelling, chemical synthesis and biological assays, will guide future efforts for the development of a treatment for Norovirus.

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## **An evaluation of the influenza vaccination service, in community pharmacies, over the past five years (2012-2017) in Wales**

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Influenza is a respiratory infection that results in numerous cases of serious illness and even deaths every year.<sup>1</sup> Prior to 2015, Wales was the only part of the UK with a national influenza vaccination scheme, allowing community pharmacists to provide free influenza vaccinations.<sup>2</sup> There are several categories eligible for a free vaccination e.g. aged over 65.<sup>3</sup> The Welsh Government has set an immunisation target of 75%<sup>4</sup> and the introduction of the scheme hoped to aid reaching this target. The aim of this study was to analyse the data collected from all community pharmacies who provided vaccinations over the last five years in Wales (2012-2017).

Secondary analysis of data was chosen as the most appropriate method of analysis for this study. All data were provided in an Excel spreadsheet by the Chief Pharmaceutical Officer, Andrew Evans, after being collated by the NHS. It was subsequently coded and exported into SPSS and further excel spreadsheets for descriptive statistical analysis. No ethical approval was sought as all raw data had been anonymised prior to commencement of the study.

The results illustrated that uptake of influenza vaccinations in a community pharmacy setting increased year on year, from 1568 provision in 2012/13 to 26,889 in 2016/17. Multiple pharmacies (six or more branches) were the most popular choice of setting with an average of 78.4% of people choosing to access them over independent pharmacies. Individuals over the age of 65 received 55.9% of the total number of vaccinations, making them the highest eligibility category immunised. The most popular reason, with 38.9% of the responses, for receiving a vaccination at a community pharmacy was due to not needing to make an appointment.

This study concluded that the influenza vaccination service in community pharmacy is being accessed, however, pharmacies are still only providing around 4% of the total number of vaccination<sup>4</sup>. With possible further work and research into motivation for accessing the service, it could be utilised further.

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## Design and synthesis of novel CYP51 inhibitors as therapeutics for *Candida* infections

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*Candida* infections are a major problem that healthcare faces. Infections can be superficial and treated topically, however more serious infections are life-threatening and require systemic treatment.<sup>1</sup> CYP51 (cytochrome P450 lanosterol 14 $\alpha$ -demethylase) is an enzyme involved in the biosynthesis of ergosterol in the *Candida* species, an integral component in the fungal cell membrane.<sup>2,3</sup> The azole class of antifungals compete with lanosterol, preventing synthesis of ergosterol.<sup>1,4</sup> There are challenges in targeting CaCYP51, such as azole-resistant and multi-drug resistant strains of *Candida* species.<sup>1,2</sup> The aim of this project is to design and synthesise novel inhibitors of the fungal CYP51 enzyme in the *Candida* species.

Three compounds that contained imidazole groups were chosen for investigation. The compounds were docked in the active site of CaCYP51 using a molecular modelling program, and their binding potential compared with a known azole inhibitor, posaconazole. Compounds **5a** and **5d** were synthesised in three steps: an amidation reaction, a mesylation reaction and a nucleophilic substitution reaction that replaced the mesyl group with an imidazole ring. Compound **12** required an extra two preparation steps to extend its length and remove the Boc group. Successful final compounds were sent for biological testing at Swansea University.

Molecular modelling showed all three compounds to have promising docking results with CaCYP51. Only the chemical synthesis of **5d** was successful, and biological testing showed that it had poor effectiveness as an inhibitor when compared against fluconazole (IC<sub>50</sub> = 6.97  $\mu$ M, MIC = >16  $\mu$ g/mL). Synthesis steps require optimisation, such as the addition of molecular sieves to the amidation step.

The chosen series of inhibitors was successfully investigated by molecular modelling. Since only one final compound was successfully synthesised, there is plenty of scope for future work synthesising the other inhibitors and investigating their biological activity.

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## Evaluation of the discharge medicines review (DMR) service

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Discharge medicines review (DMR) service is an enhanced service<sup>1</sup> that is provided in community pharmacies across Wales. It was introduced in 2011 to address the many issues that patients faced with medication and information when they are discharged from a care setting.<sup>2</sup> The DMR service aims to overcome the issues such as: communication between healthcare professionals and resolving discrepancies in patient's medication. The aim of the project was to evaluate the usage of this service from April 2013 – August 2017 and to compare the results to the original evaluation of the service which took place in 2014.<sup>4</sup>

DMR data ranging from April 2013 – August 2017 was obtained via NHS Wales Shared services. The data was available in the form of Microsoft Excel® and was transferred to Statistical Package for Social Sciences version 23 (SPSS®). Five students undertook this project and therefore the data was evenly split between the students to enter into the SPSS software and then to quality assure it. Each student individually analysed the whole dataset.

Several key results were found such as: the number of DMRs have increased each year from April 2013 – August 2017 ( $p < 0.05$ ). From the data available on the discharge rate across Wales<sup>4</sup>, 1.6% of discharges have resulted in a DMR being completed from April 2013 – August 2017. The rate of discrepancies per prescription across Wales was 1.14 which was similar to the 2014 report.<sup>3</sup> The service has a greater uptake in Cwm Taf University and Betsi Cadwaladr University Health Boards compared to other Health Boards in Wales.

In conclusion, although the DMR service is slowly increasing in its usage with time; it is not being utilized to its full potential. Further work needs to be undertaken to understand the reasoning behind these results.

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## Evaluation of a Community Pharmacy-based Asthma Care Plan

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Asthma is mainly treated via the use of inhaler devices. Poor inhaler technique prevents patients from treating their asthma effectively, with up to 92% of patients using inhalers incorrectly.<sup>1</sup> This leads to sub-optimal drug deposition in the lungs leading to reduced effectiveness of treatment. The study aims to assess both inhaler technique and levels of asthma control.

The study recruited 61 asthmatic patients from several community pharmacies in South Wales by convenience sampling. Participants completed an asthma control test (ACT) to determine if their asthma was controlled or uncontrolled with an additional biometric data questionnaire being completed. A Vitalograph aerosol inhalation monitor (AIM device)<sup>2</sup> was used to evaluate participants inhaler technique. Ethical approval was granted by Cardiff School of Pharmacy and Pharmaceutical Sciences Research Ethics Committee and the study was approved as a service evaluation by the Aneurin Bevan University Health Board.

55% of the participants interviewed were found to have uncontrolled asthma (19 or less in the ACT) with an average ACT score of 18.2. 32.8% of participants were also admitted to hospital or prescribed oral steroids due to their asthma. Inhaler technique was poor throughout the different devices tested, particularly with metered dose inhalers (MDI) with 61.4% of these patients failing. Participants using dry powder inhalers (DPI) performed better than those using MDI's ( $P < 0.05$ ).

Using DPI's as opposed to MDI's could help overcome issues of poor technique seen with MDI's, however, some patients may not be able to generate the required inspiratory-flow rate to disperse the drug particles<sup>3</sup>, particularly young children and the elderly. The lack of propellant is also disadvantageous in the event of an asthma attack, where patients struggle to breathe. More work is needed to improve the overall state of asthma and reduce the number of uncontrolled patients to lower levels.

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## **Implementing technology in community pharmacy: the impact of Digital Literacy, Willingness to Change and Leadership**

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The pharmacy world has developed from a paper-based field to one that is now heavily reliant on technology.<sup>1</sup> Consequently, the importance of having a minimum level of digital literacy, a motivated willingness to change and a competent leader to lead this change - are key for technological advancement.<sup>2</sup> Investigating these characteristics of pharmacy staff is important in order to gauge what actions need to be undertaken in order to prevent a lack of advancement in this field.

In this study, a qualitative approach was taken. Focus groups were conducted with members of the community pharmacy team using a semi-structured topic guide. The first focus group consisted of five participants, and the second enlisted six participants. Each focus group lasted a total of two hours, and inductive thematic analysis was undertaken after transcribing focus groups ad verbatim.

This study concluded that there were certain barriers to implementation of technology in community pharmacy. These were mainly noted to be level of training, strong leadership which will incorporate good communication. The impact of digital literacy was very notable in influencing willingness to change towards a technological direction. However, digital literacy levels were noted to be dependent on the individual and influenced by age and experience with technology.

Further research should be completed on this topic, specifically what influences digital training. More data is also needed for what makes a strong, innovative leader and how such individuals can be trained.

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## **To what extent are Welsh medium services being utilised in community pharmacies in Wales.**

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Wales has two official languages, Welsh and English. In 2011 the Welsh Language Measure was introduced and the role of the Welsh Language Commissioner established, with the aim of facilitating and promoting the Welsh language.<sup>1</sup> In the commissioner's first report, it was published that only 29% of fluent Welsh speakers received their consultation with the pharmacist in Welsh (n=207).<sup>2</sup> Effective communication is central to the provision of effective healthcare as set out in the General Pharmaceutical Council's Standards for Pharmacy Professionals.<sup>3</sup> In April 2016 the Welsh Government added questions to the National Electronic Claims and Audit Forms (NECAF) to track the utilisation of Welsh medium services within community pharmacies. The aim of this study was to statistically analyse the number of services requested and conducted in Welsh.

Quantitative methods were used to analyse this secondary data. The data was provided on a Microsoft Excel spread sheet; from this, graphs and tables were created to give descriptive statistics. From April 2016 to September 2017, 419,607 services were conducted in Wales all of which have been analysed.

Over the first 6 months 1.9% of consultations were requested to be conducted in Welsh, in the last 6 months only 1.41% were requested. Aneurin Bevan Health Board had the lowest percentage of Welsh requests 0.05% and from those 78.12% were denied. In contrast, only 2.51% of the 4.64% requests were denied in Hywel Dda Health Board.

Several suggestions for the low uptake of Welsh medium services included, availability of Welsh language resources, patients reluctance to ask for a Welsh service and locality. Further analysis of Welsh language service provision should be considered as well as triangulation with qualitative data.

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## Investigating the effect of age, gender and Alzheimer's disease on mitochondrial protein expression

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Alzheimer's disease (AD) affects over 46 million people worldwide<sup>1</sup>, but the cause is mainly unknown. Age is the greatest risk factor. As oxidative stress has been implicated in ageing and early AD<sup>2</sup>, mitochondria are of particular interest, as these organelles represent both the main source and target of endogenous reactive oxygen species (ROS). The aim of this project was to compare protein expression of the five main mitochondrial complexes (CI-CV) in AD and non-AD brain samples, during aging, and within gender, the second largest risk factor. It was hypothesised that protein expression would be reduced in AD and with age, due to increased oxidative stress.

Human prefrontal cortex tissue was used, with AD samples having Braak staging of V-VI. Young (20-32), middle-aged (40-49) and old (70-99) samples were used for age comparisons. SDS-PAGE followed by Western Blot analysis was performed to visualize mitochondrial proteins. All proteins were normalized to GAPDH, using ImageJ software. One-Way ANOVA followed by Tukey post-hoc test or Student's t-test were used.

In AD samples, significant reductions were reported in CIII in females ( $p=0.0482$ ), and CII and CIV in male samples ( $p=0.0322$  and  $0.0074$ , respectively) when compared to controls ( $n=4$ ). There was an apparent trend for decreasing protein expression with advancing age in female samples, however, only CV expression was significant ( $p=0.0081$ ) between young vs. middle-aged and young vs. old groups ( $n=3$ ). No significant differences or apparent trends were reported in male samples across age.

The results suggest that mitochondrial protein expression is reduced in AD. However, as no oxidative markers were measured, it cannot be concluded that this was due to ROS damage. The apparent trend in females but not males across age groups suggests a gender difference to this age-related effect, which supports the possible protective role of reproductive hormones against ROS.

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## Deblistering tablets from a problem pack

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A Penarth community pharmacy reported that patients were encountering difficulty in opening Aspirin 75mg Gastro-Resistant Tablets from their blister packs. Patients requested that tablets be removed during dispensing and dispensed in traditional bottles. Aspirin, however, is moisture sensitive and these bottles are not appropriate. (1) Blister packs are designed to protect solid dosage forms from their manufacture until used by a patient. (2) The aim of the research was to investigate the effect temperature on blister pack performance and how accessible users find these blister packs.

A Zwick-Rowell materials testing machine was used to assess and measure the push-through force required to break the foil and eject tablets from the problem aspirin packs. These were stored at three different temperatures before testing: 4°C, 21°C and 35°C. A pilot trial was conducted by recruiting members of MPharm

IV and involving them in removing doses from six different over the counter (OTC) blister pack medicines, tablets and hard capsules. Then filling in a questionnaire to rank the pack in terms of build quality and ease of use.

Blister packets stored at 35°C required a force of  $53.6 \text{ N} \pm 6.58 \text{ N}$  to eject tablets in the push-through test compared to those stored at room temperature of 21°C which needed  $48.4 \text{ N} \pm 6.95 \text{ N}$ . Cooler temperatures at 4°C had little effect on the opening force and required  $48.3 \text{ N} \pm 6.47 \text{ N}$  to open. The pilot trial identified that smaller tablets are harder to eject than larger ones and hard capsules. 63% of volunteers had experienced similar patient queries regarding difficult to open packs when working in community pharmacy.

Blister pack performance is reduced when exposed to temperatures that are not recommended by the manufacturer and users find blister packs with larger tablets and hard capsules and with thinner lidding had the best performance.

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## Investigating the use of poly(methyl methacrylate)-co-vinylphenyl boronic acid particles to sense for lipopolysaccharide

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Lipopolysaccharide (LPS) is a macromolecule, containing multiple sugar molecules and a lipid moiety, found on the outer membrane of Gram-negative bacteria.<sup>1</sup> LPS acts a mediator in the pathogenesis of sepsis and similar conditions.<sup>1</sup> Sepsis incidence rates are increasing and it is estimated that the condition costs the NHS £2 billion a year.<sup>2</sup> Boronic acids are known to form reversible boronate ester bonds with diols in aqueous environments.<sup>3</sup> The aim of this study was to investigate the use of a boronic acid-containing copolymer to detect lipopolysaccharide.

Methylmethacrylate (MMA) and 4-vinylphenyl boronic acid (VPBA) were polymerised in a mixture of acetone and water using single-step surfactant-free polymerisation.<sup>4</sup> The resulting copolymer was incubated with multiple concentrations of FITC-labelled LPS, alongside controls. The monomer and solvent ratios were varied, and pH studies conducted to establish the factors influencing the binding of LPS and enable optimisation of the copolymer. The binding performance of each assay was determined using fluorescence spectroscopy.

The results of the study were largely disappointing on the whole. Only POLY C, containing 900mg MMA and 100mg VPBA, showed any considerable binding when compared to the control. Surprisingly POLY D, the pure MMA polymer, was capable of binding the most LPS when challenged with various concentrations of FITC-LPS. A greater yield of copolymer was produced as the amount of water in the solvent mixture increased. While it would be expected that greater binding would be observed in more basic environments, the result of the pH studies suggested that POLY C performed best at pH 6.

Currently poly(methylmethacrylate)-co-vinylphenyl boronic acid particles cannot be used to efficiently detect lipopolysaccharide. No consistent binding between the copolymer and the LPS was observed throughout the investigation. Further studies should be conducted for more definitive results.

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## Image and performance enhancing drugs (IPEDs) in sport: Existing knowledge and views of pharmacists in Wales

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In the past decade, the use of IPEDs has become more widespread.<sup>1</sup> Advanced level athletes have useful sources, such as their coaches, to obtain advice about IPEDs.<sup>2</sup> However, amateur athletes and the public are not aware of the legal consequences and health risks of misusing these drugs. Given their role as experts in medicines, pharmacists could potentially play an important role in educating the public on IPEDs to overcome these issues. In this study, we look into pharmacists' current knowledge on IPEDs, their confidence level in giving advice on these drugs and their interest in receiving training.

This study was granted ethical approval by the School of Pharmacy and Pharmaceutical Sciences Research Ethics Committee. A total of 494 paper-based questionnaires were sent to community, hospital and academic pharmacists practicing in Wales. The questionnaire was divided into three sections, which were the pharmacists' background, current knowledge on IPEDs and interest in education. Data was analysed using the statistical software, SPSS.

A total of 119 respondents completed the questionnaire (24.1% response rate). Only 31.9% respondents had received formal education on drugs in sport but 40.3% had been approached for advice on drugs in sport. When assessed on knowledge about prohibited over-the-counter drugs in sport, only 11.8% were able to answer all questions correctly. Only, 13.4% respondents felt confident providing advice on IPEDs to the public. A majority of the respondents (79.8%) showed interest in receiving education on IPEDs and felt drugs in sport is a public health issue (66.4%).

It appears that the public use pharmacists for advice on IPEDs but pharmacists are lacking in confidence when it comes to providing advice on this matter. Pharmacists are evidently interested in receiving education on IPEDs in sport. So, e-learning modules on IPEDs for pharmacists to complete could be developed.

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## Investigating the potential of an applicator system for reproducible manual application of microneedle patches

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Microneedles are being developed as a method of transdermal drug delivery. Microneedles penetrate the stratum corneum barrier to create channels for drug delivery to the viable epidermis, to act locally or systemically.<sup>1</sup> One advantage of microneedles is self-administration, but this would require an administration system that ensured reproducible microneedle application. The aim of this study is to develop a simple microneedle applicator that will control the forces used to apply “dummy” microneedle patches (patches without penetrative microneedles).

The study was ethically approved. A simple microneedle applicator was identified and fifty participants were recruited. Each participant and the researcher placed the “dummy” patches on their forearms and deltoids. The applicator was then used by the participant to apply a force to the patches on both themselves and the researcher. The force of each application was measured by a digital force gauge. This was repeated 3 times on each site. A reflection form was used to gain participant feedback. The data was collated and statistically analysed.

The results from this study were compared to a control study, where manual application i.e. without an applicator, was recorded. The variability in application forces was significantly reduced when using an applicator, compared to manual application. There were no significant differences between the forces

measured when comparing the participants' gender, age and previous medical training. The applicator was very well received by the participants.

The applicator was successful at controlling the forces used when applying the microneedle patch. Self-administration is likely to be more reproducible when using an applicator such as the one in this study. Further studies could measure forces using a pressure pad, could investigate other features of the applicator and should evaluate applicator function with penetrative microneedles.

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## **Developing an ex-vivo test method to assess the efficacy of perineal care washcloths for incontinence-associated dermatitis (IAD)**

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Incontinence associated dermatitis is a prevalent condition most commonly seen in the elderly and those with long term care.<sup>1</sup> The damaged skin allows opportunistic pathogens to colonize; a risk factor for pressure ulcers. There is a lack of well researched treatment options and little information on a treatment strategy.<sup>2,3</sup> Two antimicrobial wipes, a commercially available chlorhexidine 2%(CHX 2%) wipe and a wipe under development by the University of Bath (wipe B) were tested against *Staphylococcus aureus* and *Candida albicans* using an ex-vivo test method involving pig skin. The immediate and residual activity of these wipes was explored.

Pig skin samples were prepared and exposed to the microorganisms. The samples were wiped with either the CHX 2% wipe or wipe B using a drill and balance to maintain constant pressure and rotation, mimicking real life and reducing variability. After exposure to the wipe the samples were placed onto Franz cells and the remaining microorganisms were recovered with Universal Neutraliser. The recovered microorganisms were enumerated and the Log reduction of CFU/ml calculated. Suspension tests were also conducted using liquid extracted from the wipes. Residual activity was investigated where the skin samples were exposed to the wipes and incubated for 1 hour, 2 hours, 4 hours or 24 hours before being inoculated and recovering the microorganisms.

Promising activity was observed with the CHX 2% wipes with both pathogens in the ex-vivo and in-vitro tests. Mechanical removal of *C.albicans* from skin samples was observed with the control wipe and as such all wipes displayed good Log reduction of CFU/ml. The suspension tests showed that wipe B had little antimicrobial efficacy. CHX 2% shows residual activity up to four hours.

Chlorhexidine 2% wipes are effective against microorganisms that cause IAD and provide residual protection. More research should be conducted on the activity of Wipe B.

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## **Exploring technology and communication needs in a community pharmacy**

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Healthcare technology is advancing, enabling the pharmacist to dedicate more time to patient-oriented services, including medicines optimisation services.<sup>1</sup> Community pharmacy staff collaborate with healthcare professionals (HCPs) across the entire healthcare system, creating a need for information technology (IT) and communication systems support.<sup>2</sup> Effective communication improves patient outcomes and work efficiency, and reduces hospital admissions, polypharmacy and drug wastage.<sup>3,4</sup> The aim of this study is to evaluate the communication needs in community pharmacy and explore how technology may assist in bridging communication gaps.

A literature review of online databases, such as Web of Science, was conducted. Findings were used to create a topic guide, piloted in a focus group (FG) with community pharmacists (n=5). This was followed by two FGs with pharmacists (first FG n=5, second FG n=6). Inductive thematic analysis was used to derive themes and sub-themes from focus groups.

Themes derived from FG data included communication methods, importance and implications of communication, communication with other settings, and possible improvements for communication. Pharmacists' views relating to communication with patients, HCPs and internal staff were explored, and common forms of communication such as email, telephone, fax, written, patient medical record (PMR) system, and verbal were identified. Good communication between HCPs, patients and pharmacy staff was perceived to improve patient safety. Participants identified patient confidentiality as a communication barrier and thought that there is inadequate communication between healthcare settings.

This study identified a range of perceived unmet communication needs in community pharmacy including communication around monitored dosage systems, access to patient records across health settings, and connectivity of pharmacy IT systems. Communications and hospital discharges should have a uniform approach and standardised format to collect relevant information. All communication mediums should be secure to protect patient data.

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## Investigating application devices for microneedles to improve patient compliance and achieve consistency in force applied

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Microneedle technology was envisioned several decades ago, however the pace of research has increased in the last 10 years. Microneedles have many advantages, however a significant disadvantage includes patient reservations regarding self-application and whether it has been applied correctly.<sup>1</sup> The study aim was to use a simple applicator device on 50 individuals and to compare the forces with previous data where no applicator was used.

Fifty individuals were recruited using snowball sampling. Volunteers completed a data collection form (specifying gender, age and medical experience). They were then asked to apply a dummy microneedle using the applicator to themselves (deltoid and forearm) and to the researcher at the same sites. The forces used (Newton's) were recorded by the researcher and hidden from the volunteer. Variation in forces applied between gender and medical experience were measured in SPSS using an independent T-test, whilst age variations were analysed using a one-way ANOVA and Tukey test. A questionnaire was provided that allowed individuals to rate their experience (1-10) regarding the applicator as well as to provide feedback about their thoughts on microneedle technology.

Results obtained showed a significant improvement when using the applicator. For self-application the mean force increased from 21.61N to 27.6N with a reduction in the standard deviation from 12.44 to 4.34. Similar findings were obtained when participants applied the microneedle device to the researcher. Statistically significant variation in forces applied between males and females (p=0.01) should be investigated further. Eighty percent of those who answered the questionnaire (n=25) expressed a willingness to use microneedles in the future, contrary to prior studies which had shown patient tentativeness.<sup>2</sup>

This study has demonstrated that the use of an applicator is beneficial in improving the reproducibility of forces applied to microneedles, and consequently may increase patient confidence in self-applying microneedles.

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## Patients' perceptions, concerns and expectations of stem cell therapy in Parkinson's disease

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Parkinson's disease (PD) is a progressive neurodegenerative condition. Current first line drugs produce unwanted side effects, becoming less effective with disease progression. The lack of available disease modifying strategies, creates need for more effective therapies.<sup>1</sup> Stem cell research has led to a novel therapeutic approach in PD, which is becoming close to clinic.<sup>2</sup> Despite the benefits of patient engagement in research, there are limited studies in the field exploring small unrepresentative samples.<sup>3</sup> This project aims to uncover how PD patients perceive this approach as a future therapy and identify concerns.

A questionnaire was constructed and emailed to people in the Parkinson's UK research support network, receiving 527 responses. A preliminary analysis led to focus groups which recruited 18 participants also from the research network. Qualitative and quantitative questionnaire data was analysed using SPSS. The focus groups were transcribed, then analysed thematically.

Within themes, willingness to consider therapy and expected benefits related to stage of disease. Therapy barriers centred around side effects and surgery risk. The individuality of PD symptoms was expressed along with trust in consultant opinion. In contrast to the focus groups, questionnaire analysis found no relationship between the stage of disease and whether participants would consider therapy or trials. Participants diagnosed for 2-5 years were more likely to consider therapy if the minimum benefit was a mild improvement in motor and other symptoms. Therapy motivation varied from self-benefit to benefit of both self and others with a small proportion having purely altruistic motivation.

Despite the barriers, participants in this study were open to the possibility of considering this novel approach as a future therapy. With unrealistic expectations present among participants, further work should consider the best practice of communicating the relevant information and support to prospective PD patients of stem cell therapy and clinical trials.

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## Does UK-grown *Artemisia annua* produce Artemisinin?

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Natural products have been used throughout history<sup>1</sup> and *Artemisia annua* is an ancient Chinese herb that has found a use in contemporary society.<sup>2</sup> Artemisinin is a compound extracted from *A. annua* that has caught the attention of many, following the research by Professor Youyou Tu.<sup>3</sup> *A. annua* has been grown in different countries, other than China<sup>4</sup>, to see if more or less artemisinin is produced comparatively as a result of this environmental change. This project looks to see if UK-grown *A. annua* can produce sufficient artemisinin for extraction, leading to possible large-scale UK growth for pharmaceutical purposes.

Samples of UK-grown *A. annua* were separated into leaf, stem and flower plant material. Extraction was carried out using both, soxhlet extraction and magnetic stirrer extraction. Multiple solvents were used for their polarity: hexane, ethyl acetate and methanol. Prep thin layer chromatography was then carried out to separate the extracts into fractions so a more detailed electrospray ionisation mass spectrometry reading could be produced.

Artemisinin was successfully detected in several extracts from the leaf and flower plant materials. Positive results were found in Hexane Flower Stir 2, Ethyl Acetate Flower Stir 1 and Hexane Flower Soxhlet 3 for the flower plant material. For the leaf material, artemisinin was found in Ground Leaf Soxhlet Hexane 6,7,9, and 10. However there was only a trace quantity detected. There appeared to be a large quantity of an unknown

compound at  $m/z$  248 found throughout the plant material and in different solvent systems, which warranted further investigation.

Despite the success of extracting and identifying artemisinin from UK-grown *A. annua*, only a minimal amount of artemisinin was located. A conclusion can be made that *A. annua* is not a viable option for large-scale growth in the UK, with regards to artemisinin being the main compound required.

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## Exploring sampling methods to ensure surfaces are free of dry biofilms

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A biofilm is a complex aggregation of microorganisms encased in extracellular polymeric substances (EPS), which are attached to a surface.<sup>1</sup> Biofilms exist in wet and dry states. Biofilms are generally found in wet states although it has been demonstrated dry biofilms are on many hospital surfaces.<sup>1</sup> Bacteria within biofilms are highly resistant to antimicrobials and cause healthcare associated infections (HCAI). Fit to purpose sampling tests are needed to establish the cleanliness of hospital surfaces. The aim of the study was to establish the most effective sampling method at dry biofilm detection from hospital surfaces.

Experiments on hospital folders and keyboards were conducted. Samples were stroked with sterile swabs, three different wipes and sponges. The same pattern of sampling was used for each method and each material. A contact plate was also tested but due to its nature, not in the same way. After sampling, each material was processed using two methods. In the first method, the material was placed into a stomacher with nutrient broth, homogenised then drop-plate counted to calculate colony forming units per ml (CFU/ml) detected from the hospital surface. The second method involved placing the material into nutrient broth and observing for a turbidity change following incubation at 37C.

The contact plate and wipe 1 were ineffective at recovering bacterial cells from surfaces. Wipe 2, wipe 3 and the sponge were most effective at recovery of dry biofilm. Of these, using the stomacher method, the sponge recovered the most bacteria, followed by wipe 3 then wipe 2. Overall, the sponge was the most effective method at recovering dry biofilms from surfaces.

All materials were efficient at recovering a high concentration of biofilms from a control, however when reflecting real-world concentrations, many methods were poor. Quick and reliable methods for dry biofilm detection need to be developed to combat hospital acquired infections.

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## Optimising an iPad based app to record electronic Patient Reported Outcome Measures (ePROM) for Parkinson's disease

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People with Parkinson's disease (PwP) have a mixture of motor and non-motor symptoms (NMS), however non-motor symptoms are often overlooked and left untreated in the short appointments with the specialists.<sup>1</sup> This is a major problem as the non-declaration of these symptoms can lead to suboptimal care, an increased cost of care and a reduced quality of life.<sup>2</sup> An iPad app has been developed to help identify these NMS and provide extra information to aid the specialist and has promising initial results.<sup>3</sup> The aim of the study is to evaluate the current app and provide recommended changes in order to more effectively manage PD.

Ethical approval was obtained and 5 focus groups were conducted within South Wales. The focus groups included a mix of PwP and their carers and also participants of a similar demographic. These were recorded then later transcribed and analysed using transcript-based analysis. Inductive and deductive techniques grouped themes from all the focus groups and data was labelled, categorized and interpreted in order to identify themes. Finally a combined analysis with the previous research was completed to assess new themes and determine saturation.

Participants had a good knowledge of using technology but insufficient confidence could prove a barrier to the continued use. Data safety was a concern and some PwP were not comfortable using the iPad in the waiting room. Font size, expanding the NMS questions and additional sections were the main alterations discussed. Participant's attitudes towards technology in a healthcare environment were also reviewed.

Participants found that the iPad app could be beneficial in clinics. Participants identified some potential changes and felt additional sections could progress the app to its full potential. Further exploration of the implemented changes and additional sections is needed to ensure the app is fit for use.

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## EVALUATION OF THE DISCHARGE MEDICINES REVIEW SERVICE (DMR)

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Medication errors occur during the transition of care between different providers.<sup>1</sup> Therefore, the Discharge Medicines Review, an advanced pharmacy service was introduced in 2011. The service was developed by utilising the pre-existing Medicines Use Review framework.<sup>2</sup> Some of the aims and desired outcomes of the service are risk reduction in relation to medication errors and adverse drug events, improve communication between parties involved with care transition.<sup>3</sup> The aim of this project is to establish a new DMR baseline in relation to the previous evaluation of 2014 to identify areas for service improvement.

Data provided by the National Electronic Claim and Audit Forms from DMR payment claims was used to perform secondary data analysis. The data was imported into SPSS, quality assured and then analysed descriptively to identify trends as well as changes at times when amendments to the service were introduced. Statistical analysis was then applied to determine whether the identified changes were significant.

Since 2013, 37425 DMRs were carried out in Wales. The monthly numbers have steadily but slowly increased. Furthermore, approximately 20-30% of contractors are making claims although almost all contractors in Wales are accredited. The data does not contain information regarding individual contractor claims, therefore it is difficult to determine the reasons for the lack of engagement.

In conclusion, the DMR service is still underutilised by healthcare providers. The reasons for this cannot be determined by the current available data, however it can be suggested that future engagement with contractors in the form of interviews and questionnaires will identify these reasons. Furthermore, the introduction of an electronic system has shown to have a significant effect on the DMRs completed. It shows a promising increase in engagement with the service which has the potential to enhance the health and wellbeing patients during times when NHS resources are limited.

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The following abstracts have been withheld as they have been or will be published elsewhere, and/or due to intellectual property or confidentiality issues:

### **An evaluation of the emergency hormonal contraception enhanced service use over the last 5 years via the specified PGD in community pharmacies across Wales**

Sarah Alzetani, R Hayward and E Mantzourani

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### **Binary drug loaded contact lenses to treat acanthamoeba keratitis**

Laura Emily Bolger and CM Heard

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### **Targeting zinc transporters to inhibit breast cancer cell division**

Nawshin Chowdhury and KM Taylor

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### **A Drug-Loaded Thermosetting Hydrogel to Treat Alveolar Osteitis**

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### **The Development of Mucoadhesive Films to Treat Periodontitis**

Mali Dafydd, CM Heard and EL Board-Davies

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### **Exploring the characterisation, biological function and therapeutic potential of receptor clustering**

Emily Doe and AT Jones

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### **An Evaluation of Emergency Hormonal Contraception Supply via a Patient Group Direction, as Part of an Enhanced Service in Community Pharmacies in Wales, Over the Past 5 Years**

Rebecca Hayward, S Alzetani and E Mantzourani

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## **The reconstitution of functional protein from cell membrane fragments into droplet interface bilayers**

Lydia Holloway and OK Castell

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## **Comparing HER family receptor levels, activation, and signalling in HER2<sup>+</sup> and trastuzumab resistant breast cancer**

Ruth Jones, JM Wymant and AT Jones

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## **Serum starvation and *in vitro* cell models of drug delivery: where is the consistency?**

Abbey Lloyd and AT Jones

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## **Design and manufacture of a fluorescent Nano-pH probe for cell biology and drug delivery research**

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## **Development of a 3D Printing Platform for Tissue Bioprinting**

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## **Optimization of print parameters to develop 3D-bioprinting using microfluidics**

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## **Targeting zinc transporters to inhibit breast cancer cell division**

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## Exploring the tumour-stromal interactions to identify novel clinical markers for prognosis and therapeutics in colorectal cancer

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Colorectal cancer remains one of the most common cancer types in the UK, accounting for over 16,000 deaths every year. Although advancements have been made with the production of targeted therapies, treatment still relies heavily on chemotherapeutics. The tumour microenvironment (TME) is becoming an increasingly popular target for novel clinical targets due to its significant role in carcinogenesis. Several markers have been selected to validate any tumour-stromal interactions in tissue and RNA samples from colorectal patients. This was determined with the use of immunohistochemical staining and gene expression analysis. Collagen expression was found to be significantly higher in the stromal compartment than in the epithelial (p value < 0.0001). PTEN analysis of two cohort studies showed contradicting results and CD26, ZFP64 and CLIC3 had different levels of expression in the stroma although they were not significant. 3D organoid culture models of colorectal cancer were then used utilised to decipher the role of collagen in the stroma. Increasing collagen I concentrations in the Matrigel did not affect cell growth. These results indicate some role for stromal interactions in tumourigenesis and invasion. Further work will need to be conducted to truly validate these results and possibly identify other clinical markers related to TME regulation for the treatment of colorectal cancer.

## Src family kinases as regulators of breast cancer cell migration.

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1 in 8 women are diagnosed with breast cancer in England and Wales, and breast cancer death rates are increasing despite the advances in treatments. Aggressive breast cancers, particularly triple- negative breast cancers (TNBC) and endocrine resistant cancers raise a lot of concerns as we face challenges in treating those cancer types. Src family kinases (SFKs) are non-receptor tyrosine kinases that phosphorylate various proteins and play a major part in cellular signaling pathways such differentiation, migration, invasion and proliferation. TNBC and endocrine resistant breast cancers were established to have elevated expression levels of SFKs which contribute to their aggressive and metastatic phenotype. Therefore, the aim of this study was to investigate whether metastasis is governed by individual SFKs, particularly Src, Lyn and Fyn, or whether it is a phenotype that is caused by combined effects of the three SFKs. This was established by testing the effects of short-interfering RNA (siRNA) and the broad Src inhibitor AZD0530 on SFK gene and protein levels, on the FAK and MAPK protein levels, and also on the proliferation and migration in three cell lines (MDA-MB231, MDA-MB468, and a 3-year Tamoxifen-resistant cell model of MCF7 known as TOYA). Wounding assays showed that AZD0530 was able to induce migration inhibition in MDA-MB231 in a dose dependent manner, with no effect on cell proliferation. Furthermore, Western blotting showed that AZD0530 had a dual action by decreasing phosphorylated Src total Lyn protein expression, while Fyn levels remained unchanged. In contrast, siRNA treatments was able to knockdown individual SFKs however, Fyn was not fully silenced. Moreover, cell migration in the MDA-MB231 treated with siRNA was not affected, which could be due to maintained levels of phosphorylated FAK and MAPK, that contribute to cell survival and migration. Interestingly, Fyn siRNA caused a 10 fold increase in phosphorylated FAK expression. This lead to the conclusion that Fyn kinase could be the member of SFKs that majorly contribute to the aggressive phenotype in breast cancers, and that individual SFKs knockdown was not as effective as AZD0530 in inhibiting cell migration.

## TEAD Signalling in Endocrine Resistant Breast Cancer

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Resistance can be acquired during antihormone (AH) therapy of ER+ breast cancer culminating in relapse. VGLL1 is upregulated and growth-contributory in some novel cell models resistant to prolonged AH exposure. VGLL1 is a coactivator for TEAD1-4 transcription factors, which also interact with further coactivators regulated by the Hippo pathway. TEAD signalling has not been explored in AH resistance and this comprised the project's overall aim using AH resistant cell lines and clinical breast cancer samples.

Affymetrix microarray analysis using Genesifter and RT-PCR monitored ER and VGLL1 in luminal A and B-derived faslodex, tamoxifen or oestrogen-deprivation resistant models and also determined the most promising deregulated mRNA profiles for further VGLLs, TEAD1-4 and Hippo elements. Immunohistochemistry was optimized using a pan-TEAD antibody, applied to 93 clinical breast cancers and HScored to explore TEAD versus signalling biomarkers and clinicopathology. KM plotter determined any TEAD members related to shortened relapse-free survival (RFS) in endocrine-treated patients.

VGLL1 was induced only in luminal A-derived models that lost ER during prolonged AH. VGLL3 and TEAD1 were induced in faslodex resistant models, while TEAD4 modestly increased in further antioestrogen resistant lines. Some Hippo pathway changes (TAZ/YAP gain; tumour-suppressive element decline) were found primarily in antiestrogen resistant cells. Nuclear TEAD was expressed in most clinical samples. There were few clinicopathological associations, but TEAD correlated with growth factor signalling biomarkers (e.g. HER4, MAPK activity, Fos in ER+ and STYK1 in ER- patients respectively). TEAD 1 and 4 mRNA related to shortened RFS in luminal A patients.

This considerable deregulation of VGLL/TEAD/Hippo pathway signaling suggests a contribution to AH resistance in vitro, and also contribution to acquisition of resistance in luminal A patients possibly involving interplay with growth factor pathways. With further investigation, TEAD signaling might provide a new therapeutic target in AH resistant breast cancer.

## The effect of Hepatocyte Growth Factor, Paclitaxel (Taxol), Selenium and Gamma Linoleic Acid on Tight Junctions and Metastases of Breast and Lung Cancer

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Lung cancer and breast cancer are leading causes of cancer related death worldwide. More so than primary brain tumours, breast and lung cancer associated brain metastases are diagnosed. Therefore, the role of tight junctions in cancer metastases have been investigated with the aim to find a way to prevent breast and lung cancer associated brain metastases. The tight junction were modulated using tight junction strengthening, disrupting and chemotherapeutic agents. The expression of the tight junction proteins Claudin-1 and Zo-1 were observed to determine the tight junction morphology, because these proteins are normally expressed when tight junctions are present and strong. The weaker tight junctions get, the lower these tight junction proteins are expressed.

Tissue culture, transepithelial resistance and immunofluorescence procedures were used to carry out this study. Transepithelial resistance gave a p-value<0.05, therefore, showed significant results between treatment with agents and the modulation of tight junctions. Furthermore, immunofluorescence also showed that HGF strengthens the tight junctions by increasing the expression of claudin-1 and zo-1; Taxol protected the tight junctions without strengthening or disrupting the tight junctions, thus, maintaining stable claudin-1 and zo-1 expressions; and finally, GLA and Selenium disrupted tight junctions whereby suppressing the expression of tight junction protein claudin-1 and zo-1. This study gave only preliminary results, and further studies involving methods such as ECIS is suggested to achieve more reliable results, and the development of new brain metastases management strategies.

## Examining the Activation and Targeting of Zinc Transporter ZIP7 in Breast Cancer

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ZIP7 (SLC39A7) is a zinc transporter of the LIV-1 subfamily, it is responsible for the transportation of zinc stores in the endoplasmic reticulum. ZIP7 has also only been recorded to be located in the endoplasmic but studies of fluorescent microscopy of transfected wt ZIP7 has always shown a perinuclear region which is not normal for an ER located protein. ZIP7 has also been associated with MAPK, mTOR and PI3K pathways in breast cancer, ZIP7 overexpression along with its Mediated zinc release has also been shown to causes phosphate inhibition in aggressive forms of Anti-hormone breast cancer. High levels of Zinc in the nucleus has been linked to DNA replication which is important for mitosis in cells. Yet there is no known zinc transporter which actively transports zinc into the nucleus. Due to ZIP7s perinuclear ring our aim is to investigate ZIP7 localization on the inner nuclear membrane which would allow it access to the nucleus and it could potentially be the zinc transport for the nucleus.

We used fluorescent microscopy on three different microscopes to image the different coverslips of MCF-7 cells transfected for wt ZIP7 and tagged for ZIP7 and markers of the inner nuclear membrane. The results of the microscopy were promising with some evidence of localization of ZIP7 and important nuclear membrane bound proteins: Lamin B receptor, Lamin A/C and Emerin.

Our data shows very positive results of ZIP7 localizing in the inner nuclear membrane, but further analysis through the use of other inner nuclear membrane proteins is required or through the use of electron microscopy. The confirmation of ZIP7 being located in the Inner nuclear membrane could influence the drug discovery targeting ZIP7 in Anti-hormone breast cancer.

## Analysing a novel series of fluorinated antiandrogens for prostate cancer treatment

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Prostate cancer is androgen-dependent and a major cause of male morbidity and mortality worldwide. Moreover, better non-steroidal antiandrogens are urgently required to treat it because the current clinical standards of bicalutamide and enzalutamide have widespread resistance and only moderate antiproliferative effects. Fortunately, the insertion of lipophilic fluorine atoms can improve the binding affinity, pharmacokinetics and physiochemical properties of anti-cancer drugs. Thus, this project aimed to see if a novel series of fluorinated bicalutamide analogues were more effective at treating prostate cancer than bicalutamide and enzalutamide in vitro.

AR-dependent LNCaP cells and AR-independent PC3 cells were treated with twenty-four fluorinated antiandrogens (A-X), bicalutamide and enzalutamide at 0-100µM for 96 h in triplicate. Percentage cell viability was normalised to DMSO using MTT assays, and IC<sub>50</sub> values calculated using non-linear regression in GraphPad Prism v6.01. The effect of the most promising compounds - I, J and O -, upon relative PSA expression and the cell cycle in LNCaP cells were then analysed through qPCR and FACS after PI-staining.

Regarding LNCaP IC<sub>50</sub> values, One-Way ANOVA with Tukey's HSD post-test showed that compound I differed significantly to bicalutamide ( $p < 0.05$ ). Kruskal-Wallis with Dunn's post-test then showed that compound J differed significantly to bicalutamide ( $p < 0.01$ ) and enzalutamide ( $p < 0.05$ ). Compounds I, J and O also downregulated PSA expression during qPCR. Furthermore, compound O caused slight DNA fragmentation during FACS analysis, but did not produce a statistically significant dose- response.

Regarding PC3 IC<sub>50</sub> values, Kruskal-Wallis with Dunn's post-test showed that compound I ( $p < 0.05$ ) and compound J ( $p < 0.001$ ) differed significantly to bicalutamide, and compound J to enzalutamide ( $p < 0.05$ ).



Thus, novel fluorine modifications have made compounds I and J significantly stronger AR-antagonists than bicalutamide and enzalutamide, with an additional unknown mechanism of action due to their activity in the AR-negative PC3 cell line which should be explored in future work.

## Exploring mechanisms of Herceptin resistance induced by prolonged endocrine treatment in breast cancer

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Prolonged endocrine therapy is a mainstay treatment for oestrogen receptor-positive (ER+) breast cancer, encompassing both luminal A (ER+/ERBB2-) and luminal B (ER+/ERBB2+) patients. Unfortunately, approximately 40% of ER+ patients acquire resistance to endocrine therapy. Both luminal A and B tumours may, in some patients, be treated with Herceptin (potentially alongside further antihormones) to control such relapse. Unfortunately, however, many patients show only limited Herceptin responses. Intriguingly, prolonged endocrine treatment is capable of inducing Herceptin resistance *in vitro*, although the underpinning mechanisms remain unknown. This project aimed to identify changes in candidate growth factor (ERBB) or non-candidate signalling that may contribute to antihormone-promoted Herceptin resistance by studying prolonged endocrine-treated luminal A- (tamoxifen) and luminal B- derived (Faslodex) breast cancer cells *in vitro*.

Long-term antihormone-promoted Herceptin-resistant (TAMRLT; FASRLT) and matched Herceptin-responsive (TAMR; BT474(con)) lines were studied under basal conditions or following 7-day Herceptin. Affymetrix 1.0ST microarray analysis and RT-PCR discriminated deregulated basal mRNA expression of candidate ERBB receptors. Western blotting or ICC was used to investigate protein expression, activation, and localisation for deregulated ERBB2, ERBB4, downstream kinases, and ER. Transcriptome interrogation using bioinformatic tools was employed to identify non-candidate pathways.

Herceptin significantly downregulated ERBB2 in TAMRLT and TAMR cells; however, this was not seen in FASRLT contrasting the matched Herceptin-responsive cells. Prolonged antihormone treatment promoted a shift in ERBB signalling away from ERBB2 (TAMRLT) and ERBB3/pAKT (FASRLT) towards increased ERBB4 in both Herceptin resistant models, which also retained MAPK activity and lost ER. Moreover, non-candidate metabolic processes and NOTCH signalling were significantly induced in TAMRLT and FASRLT, respectively. The induced candidate and non-candidate signalling may feasibly underpin antihormone-induced Herceptin resistance in luminal A and B breast cancer cells. Findings from this project provide avenues for future therapeutic exploration *in vitro*, and perhaps ultimately in the clinic, with pan- ERBB and  $\gamma$ -secretase inhibitors.

## Investigating the Differential Effects of OH14+/-TRAIL in the Bulk and Stem MCF-7 Cell Population

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Breast cancer is the second leading cause of cancer-related mortality worldwide in women. Intra-tumour heterogeneity is presumed the primary cause of therapeutic resistance and disease progression. Breast cancer stem cells (bCSCs) are hypothesised to drive both carcinogenesis and intra-tumour heterogeneity. Ensuring the therapeutic elimination of these cells is essential in preventing relapse and metastasis. The inhibition of the endogenous apoptosis inhibitor; cFLIP, selectively sensitises bCSCs to TRAIL- induced apoptosis. However, this does not translate to the bulk cell population of a tumour. This study investigated whether the inhibition of the endogenous inhibitors of apoptosis proteins (IAPs) could sensitise the bulk cell population to the effects of TRAIL and a newly formulated cFLIP inhibitor, called OH14. The differential protein expression of key apoptotic components were compared between the bulk and stem population of an MCF-7 cell line using Western blot analysis. In addition, different combinations of OH14, TRAIL and SMAC mimetic were

tested on the stem and bulk population. The cell viability of the bulk population was measured by CellTite-blue assay, while the tumoursphere and colony forming capacity of bCSCs were measured with both tumoursphere and colony forming assays. The expression of cIAP1 was significantly higher in the bulk population compared to the stem population. Furthermore, the use of a SMAC mimetic sensitised the MCF-7 bulk population to OH14/TRAIL. However, contrastingly the SMAC mimetic increased both tumoursphere and colony formation.

In conclusion, the SMAC mimetic alleviated apoptosis inhibition and sensitised the bulk population to OH14/TRAIL, however enriched the stem population. These differences in cell responses to therapeutics highlight the importance in considering these differences when designing therapeutics to prevent both tumour growth and metastasis.

## Investigating the efficacy of a cFLIP inhibitor in pancreatic cancer cell lines

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Pancreatic ductal adenocarcinoma (PDAC) is a notoriously difficult disease to treat with a bleak outlook for those who are diagnosed. It progresses in a stepwise manner, which gives the opportunity for patients to be genotypically profiled and thereby treated in a targeted manner. The TRAIL pathway is upregulated in 80% of PDAC patients and is able to selectively induce apoptosis in cancer cells. TRAIL agonists have been developed but results in clinical trial have been disappointing, possibly as a result of the TRAIL pathway also causing migration, proliferation and survival. OH14 is a targeted therapy which is believed to bind to cFLIP allowing TRAIL-induced cleavage of caspase 8 and thereby initiating apoptosis. This project aimed to assess the efficacy of OH14, confirm the mechanism behind its action, clarify aspects of the TRAIL pathway and to compare the effect of OH14 and TRAIL treatments on cell migration in the surviving cell population.

Primary cell lines derived from resected pancreatic tumours were used to determine OH14 efficacy. These were selected from as potentially TRAIL responsive based on the expression levels of TRAIL-R related pathway components. Cell Titre Blue was used as an indirect indication of apoptosis in cell lines and Boyden chamber assays were used to measure migration.

OH14 resulted in a loss of viability increasingly over time when given to PDAC cells (15-23% 48h, 32-35% 72h). Furthermore, there was cross talk between the different arms of the TRAIL pathway. Apoptosis induction by OH14 was partially attributed to caspase 8 interaction, but pharmacological inhibition of caspases failed to completely rescue the loss of cell viability, suggesting that other survival-related pathways may also be affected by OH14. OH14 treatment of pancreatic cancer cells left a significantly less migratory population than TRAIL treated cells, demonstrating that this is potentially a safer treatment for patients.

In conclusion OH14 induces apoptosis in PDAC cell lines and leaves a significantly less migratory population than TRAIL treated cells, indicating its appropriateness for further research and clinical trials.

## Statins as anti-cancer agents for breast cancer

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Evidences suggest and represent statins as a novel anti-tumour therapy for many types of cancers, including breast cancer. Statins inhibit the enzyme-3-hydroxy-3-methylglutaryl-coenzyme-A- reductase (HMGCR, HMGCoAR). Also, statins have pleiotropic effects, which allow them to decrease the vascular inflammation and they can be aused in autoimmune disease, because of their immune-modulatory capacity. There are different types of statins, but this project only focused on simvastatin and pravastatin. Also, in this project, we tried to investigate the effects of statins on breast cancer cell growth, on cellular migration and on breast cancer cell signalling. MTT assay and Ki67 assay used to evaluate the viability and proliferation of triple-negative breast cancer (TNBC) cells, western blot and Protein array used to determine whether statins affected the activity of intracellular non-receptor protein kinases. Cellular migration was investigated by using Wound

Healing Assay. SPSS was used for the statistical analysis of the data collected from the experiments. Both agents inhibited cellular proliferation and migration in the two TNBC cell models with simvastatin being the more potent agent. This was accompanied by a reduction in EGFR activity. Protein array studies revealed that statin treatment also decreased expression and activity of FAK, a key protein involved in cellular migratory responses along with STAT2.

Overall, our findings suggest that statins exert anticancer activities potentially through the modulation of EGFR and other signalling pathways that control proliferation and migration. Whether this is a direct effect or through regulation of cellular cholesterol remains to be determined.

## **Investigating The Activity and Post- Translational Modifications of Zinc Transporters ZIP6 and ZIP10, during mitosis.**

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Zinc, which is the second most abundant trace element in the human body, participates in cell growth, cell division, differentiation, and development. Disrupted zinc homeostasis therefore contributes to disease, such as cancer. Only recently have we truly learnt to appreciate its participation in cell signalling and regulation of gene expression, and therefore understand its involvement in cancers of various tissue types. ZIP transporters are known to regulate zinc homeostasis, and it has recently been discovered that ZIP6 and ZIP10 of the LIV-1 subfamily regulate cell division by zinc influx. Furthermore, both transporters are associated with breast cancer spread. In this study, we report confirmation of preferential expression of ZIP6 and ZIP10 in mitotic cells, and of a second ZIP6 cleavage at the plasma membrane, by immunofluorescent staining of MCF-7 cells. To our knowledge, we are the first to allude to a complex N terminal structure of the ZIP6/ZIP10 heteromer that might reflect the previously reported ectodomain of ZIP4 homodimers. This was realised by both simultaneous, and separate incubation of cells with ZIP6 and ZIP10 N-terminal binding antibodies. Proximity ligation assay and western blotting analysis have permitted the analysis of ZIP6 phosphorylation by candidate kinases CK2 $\alpha$  and GSK3 $\beta$ , and for the first time we allude to a hierarchical phosphorylation of ZIP6, as is the case for many transmembrane receptors and channels. These findings provide greater insight into the ZIP6:ZIP10 mitotic paradigm that could be harnessed for therapeutic benefit in the future.

## **Does ADAM15A Form a Complex with c-Abl, Matriptase and Hepsin and Does It Affect ADAM15A Regulated Intramembrane Proteolysis?**

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A disintegrin and metalloprotease 15A (ADAM15A) is a type I transmembrane glycoprotein that is implicated several advanced cancers. ADAM15A is involved in the degradation of the ECM and multiple pro-survival mechanisms. ADAM15A is known to interact with intracellular proteins such as tyrosine kinases like c-Abl. The ADAM15A ectodomain is shed and the intracellular domain (ICD) undergoes regulated intramembrane proteolysis (RIP) to release a signalling peptide intracellularly. The ICD is known to be RIPed by a serine protease, matriptase or hepsin. This research project uses co-immunoprecipitation and western blotting to establish which interactions exist between ADAM15A and the serine proteases and between c-Abl and the serine proteases. It was found that ADAM15A interacted with the serine proteases. The interactions were then tested to see if they were c-Abl dependent by using a chemotherapeutic drug Nilotinib which is known to inhibit c-Abl directly. Nilotinib did not seem to have an effect on the association between ADAM15A and the serine proteases, however the association between ADAM15A and c-Abl was upregulated with the downregulation of c-Abl activity.

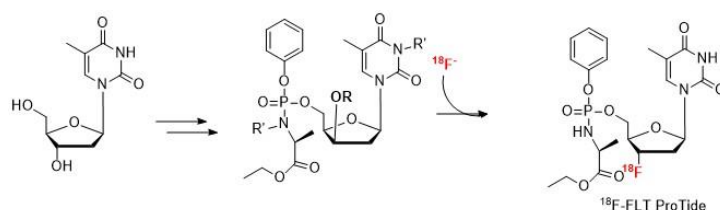
## Radiochemical synthesis of $^{18}\text{F}$ -radiolabelled ProTides for Positron Emission Tomography

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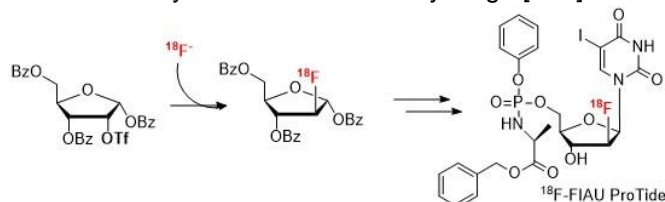
Positron Emission Tomography (PET) is a highly sensitive imaging technique used in cancer diagnosis, treatment planning and monitoring of therapy response.  $^{18}\text{F}$  is an optimal PET label considering its half-life (110 min) and imaging resolution. One of the major challenges in  $^{18}\text{F}$ -PET research is the installation of the weakly nucleophilic  $^{18}\text{F}$ fluoride into a precursor molecule to access novel  $^{18}\text{F}$ -tracers. Fluorinated nucleosides represent an important class of diagnostic probes for PET imaging as well as anticancer and antiviral therapeutic agents. However drug resistance still represents a major problem. The ProTide approach is a strategy to synthesize prodrugs of the nucleoside monophosphates which overcome their main resistance mechanisms. The challenge of the project is the  $^{18}\text{F}$ -fluorination (hot fluorination) of ProTides which may be potential new PET imaging agents and could thus represent a model system to visualize pharmaceutical effects and bioactivation of ProTides directly *in vivo*.

The pro-nucleotide multistep synthetic chemistry has been applied for the synthesis of ProTides. The  $^{18}\text{F}$ -radiolabeling of the precursor molecules was performed in an Eckert & Ziegler automated synthetic Modular Lab placed into a shielded hot cell. The radioactive reaction mixtures were analyzed by radio HPLC, and radio TLC. Two different approaches have been followed to access two chemically distinct radiolabelled ProTides. The 3'- $^{18}\text{F}$ FLT ProTide was synthesised via a late stage  $^{18}\text{F}$ fluorination of *ad hoc* synthesised precursor molecules (Figure 1).



**Figure 1:** Radiochemical synthesis of  $^{18}\text{F}$ - FLT ProTides

The 2'- $^{18}\text{F}$ FIAU ProTide was synthesised via an early stage  $^{18}\text{F}$ fluorination approach (Figure 2).



**Figure 2:** Radiochemical synthesis of  $^{18}\text{F}$ -FIAU ProTides

These radiolabelled probes could provide evidence for the *in vivo* behaviour of this class of compounds by answering key questions about their metabolism and uptake directly.

In addition, the project focused on the synthesis of two novel classes of non-radiolabelled fluorinated ProTides. A series of uridine based ProTides (FIAU ProTides) and a series of coumarin based FLT ProTides have been synthesised and evaluated for their antiviral activity and fluorescent properties respectively.

## Nutritional abnormalities in patients receiving long-term home parenteral nutrition (HPN)

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The last two decades have seen an increased drive to administer parenteral nutrition (PN) to patients in their home environments, thereby reducing associated hospital costs and improving patient quality of life. The occurrence of deranged nutritional biochemistry results has baffled PN experts for years because PN additives

are marketed for the general needs of patients and PN is tailored to each patient's requirements (both formulation and regimen). This thesis documents the investigations into HPN population characteristics, the extent of nutritional abnormalities (deficiencies and excesses) in a cohort of long term (LT) PN patients in Wales. Both cross-sectional and longitudinal retrospective study designs were employed alongside small-scale laboratory efforts to investigate stability of vitamin D in PN additives using High Performance Liquid Chromatography (HPLC). Characteristics of the HPN population in Wales were shown to be variable in terms of PN requirements for a predominantly female sample population (2:1); in whom 78.6% of patients received PN for indications relating to short bowel syndrome (SBS). A database analysis of micronutrient test results revealed a high prevalence of deficiencies of vitamin D and selenium, as well as excesses of manganese and water-soluble vitamins; which can lead to clinically relevant effects in patients. The sample population was shown to have impaired bone health since first receiving PN; respective sites of the femoral neck and total hip presented 58% and 60.8% of patients had osteopenia, while 28% and 19.6% had osteoporosis. Evidence in the literature links these clinical outcomes of metabolic bone disease (MBD) to patients' inadequate vitamin D status. A final study exploring the adequacy of the trace element (TE) preparation Additrac<sup>®</sup>, found it lacking in selenium and excessive in manganese for the general requirements of the PN population. Clinician-directed supplementation of PN outside of Additrac<sup>®</sup> was associated with better micronutrient status in patients and more test results within range.

## **The effect of L-dopa and neuroprotective agents on cell replacement therapy for Parkinson's disease.**

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Parkinson disease PD is the second most common neurodegenerative disease affecting 1.8% of population aged over 65 years. The current medications that control the symptoms of the disease are associated with limited efficacy and induction of side effects (dyskinesia) at later stages of the disease. One promising future therapy in PD is cell replacement therapy, however clinical trials declared inconsistent outcomes and developing dyskinesia related to the graft. Studies later suggested suboptimal conditions contributed on these outcomes. This thesis builds on this knowledge endeavouring to support cell transplantation therapy in Parkinson disease in models that are more closely aligned to the clinic thorough considering anti-parkinsonian medications in the model. It is addressed the low survival and efficacy problem of the transplanted cells examining neuroprotective agents that have previously shown the ability to protect nigrostriatal dopaminergic neurons against toxic challenges. In addition, this thesis characterises stem cell transplantation, the potential cell source for transplantation that can overcome the many practical and ethical issues surrounding foetal tissue. In the first part of the thesis, the investigations on finding neuroprotective agents to support graft survival and efficacy was achieved in the unilateral 6-OHDA lesioned rat model, treated with chronic L-dopa, (the gold standard anti- Parkinson medication), prior to, and following, cell transplantation. The results revealed for the first time that Glucagon Like Peptide-1 (GLP- 1) receptor agonists (exendin-4 and liraglutide) were capable of improving graft size and the motor and behaviour recovery results from peripheral administration. Importantly, this protection was affected by the presence or absence of L-dopa treatment, as exendin-4 supported the graft only in absence of L-dopa while liraglutide supported the graft only in the presence of L-dopa. While other neuroprotective agents (ghrelin and ghrelin receptor agonist) failed to support graft survival or efficacy in the same animal model. In the second part of the thesis, the characterisation of different source of stem cells derived dopaminergic neurons revealed for the first time that these cells can survive and function in the striatum of 6-OHDA rat model primed with chronic L-dopa treatment and exposed to L-dopa treatment for 16 weeks after transplantation. I show, for the first time, that these cells are capable of ameliorating L-dopa induced dyskinesia.

## **Development of analytical methods for the stability assessment of parenteral nutrition**

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Parenteral nutrition (PN) provides intravenous nutritional support to patients with reduced gastrointestinal function. A PN bag comprises the basic building blocks of the food groups: lipids, glucose, amino acids, vitamins, electrolytes and trace elements. Recently there has been an increase in demand for extended storage periods for PN bags, to ease management of an increasing home care market. Prior to a PN formulation being deemed safe for a patient, a laboratory simulation is carried out on the proposed admixture under the requested storage and administration conditions. Currently only the physical stability is assessed; physical testing provides no information on the quantity of each component remaining in the bag after storage. Consequently, there is a need for assessing the chemical stability of PN to indicate the quantity of each component that remains in the PN bag. A commonly used amino acid product, Aminoven® 25, contains 16 amino acids; this work aimed to develop a HPLC assay capable of quantifying the amino acids in an aqueous PN bag containing Aminoven® 25. Fluorescence detection was used as it is a highly selective method of detection, which was preferable due to the number of components in PN. To detect the amino acids, as they don't naturally fluoresce, derivatization was carried out using ortho-phthalaldehyde to form a fluorescing derivative. The developed assay resulted in validation of thirteen of the amino acids in Aminoven® 25. In addition, the method was shown to be unaffected by the iv presence of aqueous PN components, so this method is suitable for quantifying thirteen amino acids in aqueous PN containing Aminoven® 25. This assay can be used for assessing the stability during stability testing and confirming the quantity of amino acids after compounding for quality control release.

## **Exploring Focal Adhesion Kinase (FAK) as a therapeutic target in triple negative breast cancer**

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Triple-negative breast cancer (TNBC) is an aggressive cancer subtype that displays poor prognosis due to a lack of targeted therapies and an early pattern of spread. Recent evidence also points to a correlation between cancer "stem-like" cells (CSCs) and the inherently aggressive traits of TNBC. As such, targeting signalling pathways which support metastasis and CSC populations may represent an important therapeutic strategy to treat these tumours and improve current patient outcomes. The non-receptor tyrosine kinase FAK (focal adhesion kinase) is known to influence cancer development and progression, with its upregulation common in several cancer types. Indeed, FAK can regulate various cellular processes associated with disease progression including, cell survival, migration and stem-like behaviours. Therefore, we explored the influence of FAK in TNBC cells and the potential benefit of its targeting in this subtype. Whilst assessment of FAK expression and activity across a panel of breast cancer cell lines representing the major clinical subtypes revealed that FAK was not significantly augmented in MDA-MB-231 cells (model of TNBC) versus other models, MDA-MB-231 cells displayed a FAK-dependent migratory and invasive behaviour involving FAK-mediated activation of Akt and STAT3. These observations also extended to cell proliferation, with pharmacological or genetic FAK inhibition leading to perturbed cell cycle progression. Whilst FAK did not contribute to the maintenance of a CSC subpopulation, FAK was necessary for their anoikis resistance and mammosphere self-renewal, the latter regulated by FAK-dependent modulation of  $\beta$ -catenin through GSK3 $\beta$  and interaction between the FAK/Wnt signalling pathways. Using computational modelling, several novel FAK inhibitors that targeted FAK kinase-independent scaffolding function were developed and screened to assess in vitro efficacy in TNBC cells. Of all 45 compounds, 'compound 9' showed significantly improved ability to reduce cell proliferation and migration versus the lead compound, chloropyramine. As expected, this agent had little effect of FAK phosphorylation but appeared to reduce focal-adhesion targeting and subcellular distribution of FAK and significantly inhibited cell migration and growth. Our in vitro data support a case for FAK as a promising therapeutic target in TNBC with an ability to suppress both tumorigenic events and those associated with metastasis. Targeting FAK scaffolding function may represent a novel approach to developing FAK inhibitors that can circumvent resistance traditionally associated with kinase inhibitors.

## Exploring the impact of anti-oestrogen resistance on the capacity of breast cancer cells to modulate bone cell function and their sensitivity to bisphosphonates

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Acquired resistance to endocrine therapy is a major limiting factor for their clinical effectiveness, resulting in disease relapse and an associated poor prognosis. Acquired resistance is also associated with the development of an invasive and migratory phenotype in vitro that may promote metastatic spread in vivo of which bone is the most frequent site. However, it is not currently known whether endocrine resistance affects the ability of breast cancer cells to modulate bone cell function important in establishing bone metastases or whether a resistant phenotype alters sensitivity to agents commonly used to treat bone metastasis such as bisphosphonates. Thus, this thesis aimed to explore the bone cell modulatory function of endocrine resistant and sensitive breast cancer cells along with their sensitivity to the bisphosphonate, zoledronic acid. This thesis demonstrated that breast cancer cells were able to directly induce osteoclast differentiation from both murine and human precursor cells. Importantly, this effect was more prevalent in tamoxifen resistant and triple negative breast cancer subtypes. Our data also suggested that the breast cancer-mediated osteoclastogenic effect involved Src kinase, whilst bisphosphonates acted as anti-tumour agents in tamoxifen resistant cells through inhibition of EGFR/AKT/mTOR pathway. In conclusion, this thesis suggests that acquisition of endocrine resistance confers a bone modulatory ability to breast cancer cells that may contribute to the development of bone metastases. However, this thesis reports the novel finding that acquired endocrine resistance augments the sensitivity of breast cancer cells to bisphosphonates, thus representing an opportunity to target resistant disease clinically.

## Defining endocytic pathways to characterise the cellular uptake of extracellular vesicles

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There is a need for vectors that, with high efficiency, can deliver small and macromolecular therapeutics into cells and defined intracellular locations. Extracellular vesicles (EVs), including exosomes, are naturally derived nanovesicles generated in and released by numerous cell types. As extracellular entities they have the capacity to interact with neighbouring cells and distant tissues and affect physiological processes as well as being implicated in numerous diseases including tumorigenesis and neurodegeneration. They are also under intense investigation as delivery vectors for biotherapeutics. The ways in which EVs interact with recipient cells to influence cell physiology and deliver a macromolecular payload are at the early stages of exploration, but are believed to involve endocytosis. For endocytic characterisation a significant challenge is having the ability to label exosomes directly or indirectly with fluorescent probes to qualitatively and quantitatively monitor exosome-cell interaction and uptake without compromising functionality. In this thesis, techniques to inhibit different pathways of endocytosis were investigated and further developed order to establish high content in vitro platforms to study the uptake mechanisms of potential drug delivery vectors focusing here exosomes as a model system. These techniques involved siRNA depletion of prospective endocytic proteins and chemical inhibitors of endocytosis. A simple and rapid method for fluorescent labelling purified Du145 exosomes covalently was for the first time utilised to allow comprehensive analysis of the 2 cellular uptake of differentiation competent prostate cancer derived EVs in live cells using confocal microscopy. For endocytosis analyses, depletion of key endocytic proteins and the use of chemical inhibitors (Dynasore, EIPA, Rottlerin and IPA-3) indicated that a fluid phase endocytosis and/or macropinocytosis-like mechanism dependent on dynamin were involved in exosome internalisation. Over a period of six hours exosomes were observed to increasingly colocalise with lysosomes, indicating a possible intracellular terminus with significance with respects to utilisation of exosomes as drug delivery vectors. Overall this labelling method, when used in conjunction with established models of endocytic inhibition, provides new opportunities for analysing the cellular dynamics of exosomes and other extracellular vesicles as biological entities affecting cell and whole body physiology as well as investigating their potential as drug delivery vectors. The work also further enhances our ability to study multiple endocytic pathways as potential routes for entry of other drug delivery

vectors to gain important information towards the design of more efficient formulations targeting a number of diseases.

## **In-vitro characterisation of targeting ligands for enhanced delivery across the blood-brain barrier**

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The blood-brain barrier (BBB) is the most extensive and restrictive barrier to brain delivery for therapeutic agents. A low proportion of low molecular-weight agents can cross into the CNS. This decreases further as the molecular weight increases, meaning therapeutic antibodies, oligonucleotides and other supramolecular entities effectively cannot reach therapeutic levels within the CNS. Targeting ligands against receptors thought to undergo transcytosis across the brain microvascular endothelial cells (BMECs), can boost CNS delivery of therapeutics. Understanding these mechanisms, in an in-vitro setting, has proved challenging, due to the constraints of cell culture systems and the difficulty to replicate the in-vivo environment. With even the most extensively studied targeting receptor, transferrin receptor, not producing clear evidence to suggest the occurrence of transcytosis. To understand in-vitro trafficking of brain targeting ligands a pulse-chase assay, in combination with sub-cellular localisation microscopy was developed and compared to the current permeability-based assay method. The characterisation was done by comparison of transferrin receptor ligands; native holo-transferrin, the 8D3 antibody and a low-affinity variant; with the non-specific uptake probe, dextran. The method could distinguish between the two endocytosis methods, with concentration-dependent efflux efficiency observed with the targeted probes. The combination of techniques was then applied to the novel targeting ligand, Rabies-Virus Glycoprotein (RVG) peptide, to assess its suitability as a brain delivery. Studies were performed to confirm the target receptor of the RVG peptide, including competitive uptake, siRNA knockdown methods. The RVG peptide demonstrated desirable delivery characteristics, and the target receptor was confirmed as the  $\alpha 7$  nicotinic acetylcholine receptor. Finally, attempts were made to develop a total internal reflection fluorescence (TIRF) microscopy assay for the assessment of ligand arrival at the basolateral membrane of BMECs. Initial work for this was performed with the transferrin receptor and transferrin, using both labelled ligand and photoswitchable receptor constructs. In summary, the pulse-chase assay provides a complementary technique to permeability assays for the assessment of brain targeting ligand trafficking in BMEC cell-lines in-vitro.

## **Design and synthesis of novel CYP24A1 inhibitors**

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CYP24A1 (25-hydroxyvitamin D-24-hydroxylase) is a useful enzyme target for a range of medical conditions including cancer, cardiovascular and autoimmune disease, which show elevated CYP24A1 levels and corresponding reduction of calcitriol (the biologically active form of vitamin D). Calcitriol has antiproliferative and pro-differentiating properties, however use of calcitriol as a therapeutic drug is limited by hypercalcaemia. An alternative approach is the use of CYP24A1 inhibitors to prevent the metabolism of calcitriol. The aim of this research is to design and synthesise novel inhibitors of CYP24A1 to enhance the endogenous levels of circulating calcitriol. Furthermore, it is important to develop compounds that are selective for CYP24A1 over CYP27B1 so that the generation of calcitriol itself is not blocked. In order to understand the requirements of inhibitor binding to the enzyme-active site, it would be useful to have a 3D structure of both human CYP24A1 and CYP27B1. However, to date, no human crystal structures are available for either of these enzymes. Therefore, a homology model for CYP24A1 has been developed and published. A CYP27B1 homology model was developed, using a combination of homology modelling, molecular dynamics simulations, and molecular docking to understand the satisfactory explanation of the binding selectivity of the CYP27B1 model with the natural substrate and with selective inhibitor complexes. Docking results for CYP27B1 showed amino acids Arg107, Asn387 and Asp320 have an important role in binding interactions to form hydrogen bonds with inhibitors. The development of potent and selective inhibitors from threeazole series was investigated. Development of series one using pyridine, imidazole and triazole as the haem binding group was synthesised successfully. The compounds exhibited weak potency and  $IC_{50}$  ranging between 10.2 to 28.4  $\mu M$  against



CYP24A1. Owing to the low CYP24A1 inhibitory activity the compounds were not evaluated against CYP27B1. Series two, bis(3-methyl-1-phenyl-1H-pyrazol-5-yl) derivatives, was synthesised successfully. Two compounds were moderate CYP24A1 inhibitors and so were further evaluated against CYP27B1. However, these compounds showed enzymatic inhibition ( $IC_{50} = 0.57 \mu M$  and  $0.41 \mu M$ ) against CYP27B1, that is they were more selective for CYP27B1, which could be rationalised from docking experiments. A series of (*E*)-N-(2-(1H-imidazol-1-yl)-2-(phenylethyl)-3/4-styrylbenzamides have been synthesised using an efficient synthetic route and shown to be potent inhibitors of CYP24A1 ( $IC_{50}$  0.11 - 0.35  $\mu M$ ) compared with the standard ketoconazole. Molecular modelling using our CYP24A1 homology model showed the inhibitors to fill the hydrophobic binding site, forming key transition metal interaction between the imidazole nitrogen and the haem  $Fe^{3+}$  and multiple interactions with the active site amino acid residues.

## Detection of prostate cancer biomarker using molecularly imprinted polymers.

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Successful treatment of prostate cancer (PCa) depends on early diagnosis and screening, which currently relies on the measurement of serum prostate specific antigen (PSA) levels. The overarching aim of the project was to generate molecularly imprinted polymers for PCa biomarkers, with subsequent integration with a sensing platform to allow for rapid, point-of-care detection and monitoring. The initial work involved the use of simple PSA epitopes for epitope imprinting using conventional imprinting techniques. A four amino acid sequence from the C-terminus of PSA was imprinted with methacrylic acid (MAA), acrylamide (Aam) and urea monomers to obtain bulk imprinted polymers. Apparent  $K_d$  of 102  $\mu M$ , 154  $\mu M$ , 194  $\mu M$  were obtained for MAA, AAm and urea based bulk mini-MIPs respectively. Epitope imprinting was further developed using a surface imprinting approach, via electropolymerisation of dopamine to detect an epitopic sequence from pro-PSA. An improvement in  $K_d$  from bulk-imprinted polymers, with an apparent  $K_d$  of 2.9  $\mu M$  was obtained with the surface electrochemical MIP sensor. However, both epitope imprinting techniques lacked the requisite sensitivity to enable measurement of clinically relevant concentrations of PSA (nM range). As a consequence, a more sophisticated technique called "hybrid" imprinting was developed to build an electrochemical MIP sensor. The hybrid imprinting approach utilised an aptamer with established affinity towards PSA in combination with a polydopamine electropolymer to imprint PSA. The resulting aptamer lined polymer pockets exhibited high selectivity and affinity towards PSA (apparent  $K_d$  0.3 nM). The apta-MIP sensor was also able to discriminate between PSA and a homologous protein (human Kallikrein 2) and was resilient to fouling from serum proteins. The apta-MIP sensor was further translated to a MOSFET device whereby successful detection of PSA at clinically relevant concentration was obtained in human plasma.

## Mechanisms of virucidal action of alcohol and metallic ions against nonenveloped viruses

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Studying the mechanism of action (MoA) of biocides against pathogenic microorganisms is crucial to understand their efficacy and limitations, and to develop more efficient microbicidal formulations. Combining alcohol and zinc has been reported to enhance microbicidal activity, but the reasons for such activity are unknown. This study focuses on the impact of combining ethanol and zinc salt at pH 10.5 against nonenveloped viruses. The study is focused on three different aspects: i) virucidal activity screening of ethanol:zinc combinations against bacteriophages and human viruses; ii) impact of ethanol:zinc combinations on virus structure, particularly the viral capsid and nucleic acid, using Transmission Electron Microscopy (TEM); Atomic Force Microscopy (AFM) and agarose gel DNA electrophoresis and iii) chemical speciation and stability of ethanol:zinc combinations over time. The combination of ethanol with zinc salt was found to be more effective against viruses than control formulations containing sole active ingredients and/or excipients only. Activity test of 40%(w/v) ethanol with 0.1% (w/v) zinc salt with excipients (RB- 002 formulation) against F116 and adenovirus type 2 (AdV2) at 60 min contact time yielded  $0.68 \pm 0.02$  and  $5.26 \pm 0.10 \log_{10}$  reduction, respectively. In comparison, 0.1% (w/v) zinc salt only with excipient (RB-002G formulation) showed no virucidal activity against bacteriophage F116 ( $0.14 \pm 0.02 \log_{10}$  reduction) and AdV2 ( $0.80 \pm 0.12 \log_{10}$  reduction) in

suspension. Differences between activities against bacteriophage MS2 and poliovirus type 1 were similar as the ones found between F116 and AdV2. Formulation containing 40%(w/v) ethanol with 0.1% (w/v) zinc salt produced a range of structural damage to F116 and attP AdV5 indicating possible capsid alteration. Effect of the combined formulation on viral capsid was confirmed with AFM with a possible decreased in virus capsid stiffness and significant virus capsid height reduction over 10 min contact time. F116 DNA damage was detected upon exposure to 40%(w/v) ethanol with 0.1% (w/v) zinc salt with excipients, but no damage was detected on AdV2 DNA through electrophoresis analysis. The alcohol/zinc formulation system at pH 10.5 was shown to have promising virucidal activity against non-enveloped viruses at room temperature following an alteration of the viral capsid, and possible damage to the viral nucleic acid. This study also showed the limitations of using bacteriophage as surrogate for mammalian viruses.

## **A mixed methods approach for assessing student and staff perceptions and experiences of a new collaborative transnational pharmacy programme**

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This doctoral thesis reports on a longitudinal, mixed methods investigation of staff and students' views, expectations, and experiences of a collaborative pharmacy programme between Cardiff University School of Pharmacy and Pharmaceutical Sciences (CU) and Taylor's University School of Pharmacy (TU). Despite a growing body of empirical research on transnational staff and students' expectations and experiences, longitudinal mixed methods studies are rare. This study combined a qualitative interview-based and focus group approach with a quantitative questionnaire-based method. The overall aim is to gain a better understanding of the teaching and learning experiences of staff and students in a transnational education (TNE) programme. The qualitative element explored staff expectations and experiences in the early stage of the collaborative programme while student expectations and experiences were investigated at different points in time throughout their 4-year pharmacy study. The quantitative element investigated and compared the learning environment perceived by participating students in TU and CU. Data collection took place over a period of 36 months and comprised four phases. In Phase 1, staff and students' initial expectations and experiences of a new collaborative pharmacy programme were explored using staff interviews and student focus groups. In Phase 2, a sample of students from CU and TU were recruited to participate in a questionnaire study to assess students' perceived learning environment. In Phase 3, a number of studies were carried out using focus groups in order to find out students' pre-arrival expectations and post-arrival experiences. Phase 4 involved a self-administered questionnaire with graduate students to assess students' opinions about their overall experiences at the universities. The study revealed staff and students' expectations and their actual experiences in relation to the delivery of a transnational education. It was found that those students who participated were able to cope with sociocultural adjustment in a new learning environment. The study also provided indications of the need for training and professional development for staff to teach in a transnational environment. Finally, Malaysian students who come from a teachercentred pedagogy background should be informed and trained earlier before their transfer to lessen the impact brought about by intercultural differences in teaching and learning.

The following abstracts have been withheld as they have been or will be published elsewhere, and/or due to intellectual property or confidentiality issues:

**Design, synthesis and evaluation of novel c-FLIP inhibitors in order to sensitise breast cancer cells and breast cancer stem cells to TRAIL**

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**Antimicrobial drug LbL-assembled delivery system for orthopaedic nanocomposite bone cements.**

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